

List of contents

Ser.	Content	Page no.
1	Figure S1. LC/MS spectrum of compound 3b .	5
2	Figure S2. ^1H -NMR spectrum of compound 4b in DMSO- <i>d</i> 6 at 400MHz.	6
3	Figure S3. ^{13}C -NMR spectrum of compound 4b in DMSO- <i>d</i> 6 at 100MHz.	6
4	Figure S4. LC/MS spectrum of compound 4b	7
5	Figure S5. LC/MS spectrum of compound 4c .	7
6	Figure S6. ^1H -NMR spectrum of compound 4d in DMSO- <i>d</i> 6 at 400MHz.	8
7	Figure S7. ^{13}C -NMR spectrum of compound 4d in DMSO- <i>d</i> 6 at 100MHz.	9
8	Figure S8. LC/MS spectrum of compound 4d	10
9	Figure S9. ^1H -NMR spectrum of compound 4e in DMSO- <i>d</i> 6 at 400MHz.	10
10	Figure S10. ^{13}C -NMR spectrum of compound 4e in DMSO- <i>d</i> 6 at 100MHz.	11
11	Figure S11. LC/MS spectrum of compound 4e	12
12	Figure S12. ^1H -NMR spectrum of compound 6a in DMSO- <i>d</i> 6 at 400MHz.	12
13	Figure S13. ^{13}C -NMR spectrum of compound 6a in DMSO- <i>d</i> 6 at 100MHz.	13
14	Figure S14. LC/MS spectrum of compound 6a	14
15	Figure S15. LC/MS spectrum of compound 6b	14
16	Figure S16. ^1H -NMR spectrum of compound 6c in DMSO- <i>d</i> 6 at 400MHz.	15
17	Figure S17. ^{13}C -NMR spectrum of compound 6c in DMSO- <i>d</i> 6 at 100MHz.	16
18	Figure S18. LC/MS spectrum of compound 6c	17
19	Figure S19. ^1H -NMR spectrum of compound 6d in DMSO- <i>d</i> 6 at 400MHz.	18
20	Figure S20. ^{13}C -NMR spectrum of compound 6d in DMSO- <i>d</i> 6 at 100MHz.	19

21	Figure S21. LC/MS spectrum of compound 6d .	19
22	Figure S22. ^1H -NMR spectrum of compound 6e in DMSO- <i>d</i> 6 at 400MHz.	20
23	Figure S23. ^{13}C -NMR spectrum of compound 6e in DMSO- <i>d</i> 6 at 100MHz.	20
24	Figure S24. LC/MS spectrum of compound 6e .	21
25	Figure S25. ^1H -NMR spectrum of compound 6f in DMSO- <i>d</i> 6 at 400MHz.	22
26	Figure S26. ^{13}C -NMR spectrum of compound 6f in DMSO- <i>d</i> 6 at 100MHz.	23
27	Figure S27. LC/MS spectrum of compound 6f .	23
28	Figure S28. ^1H -NMR spectrum of compound 7a in DMSO- <i>d</i> 6 at 400MHz.	24
29	Figure S29. ^{13}C -NMR spectrum of compound 7a in DMSO- <i>d</i> 6 at 100MHz.	24
30	Figure S30. LC/MS spectrum of compound 7a .	25
31	Figure S31. ^1H -NMR spectrum of compound 7b in DMSO- <i>d</i> 6 at 400MHz.	25
32	Figure S32. ^{13}C -NMR spectrum of compound 7b in DMSO- <i>d</i> 6 at 100MHz.	26
33	Figure S33. LC/MS spectrum of compound 7b .	27
34	Figure S34. ^1H -NMR spectrum of compound 7c in DMSO- <i>d</i> 6 at 400MHz.	28
35	Figure S35. ^{13}C -NMR spectrum of compound 7c in DMSO- <i>d</i> 6 at 100MHz.	28
36	Figure S36. LC/MS spectrum of compound 7c .	29
37	Figure S37. ^1H -NMR spectrum of compound 7d in DMSO- <i>d</i> 6 at 400MHz.	29
38	Figure S38. ^{13}C -NMR spectrum of compound 7d in DMSO- <i>d</i> 6 at 100MHz.	30
39	Figure S39. LC/MS spectrum of compound 7d .	30

40	Figure S40. ^1H -NMR spectrum of compound 7e in DMSO- <i>d</i> ₆ at 400MHz.	31
41	Figure S41. ^{13}C -NMR spectrum of compound 7e in DMSO- <i>d</i> ₆ at 100MHz.	31
42	Figure S42. LC/MS spectrum of compound 7e .	32
43	Figure S43. ^1H -NMR spectrum of compound 7f in DMSO- <i>d</i> ₆ at 400MHz.	32
44	Figure S44. ^{13}C -NMR spectrum of compound 7f in DMSO- <i>d</i> ₆ at 100MHz.	33
45	Figure S45. LC/MS spectrum of compound 7f .	34
46	Figure S46. ^1H -NMR spectrum of compound 9a in DMSO- <i>d</i> ₆ at 400MHz.	34
47	Figure S47. ^{13}C -NMR spectrum of compound 9a in DMSO- <i>d</i> ₆ at 100MHz.	35
48	Figure S48. LC/MS spectrum of compound 9a .	35
49	Figure S49. ^1H -NMR spectrum of compound 9b in DMSO- <i>d</i> ₆ at 400MHz.	36
50	Figure S50. ^{13}C -NMR spectrum of compound 9b in DMSO- <i>d</i> ₆ at 100MHz.	36
51	Figure S51. LC/MS spectrum of compound 9b .	37
52	Figure S52. Tubulin polymerization assay for compound 4b	37
53	Figure S53. Tubulin polymerization assay for compound 4a	38
54	Figure S54. Epidermal growth factor receptor (EGFR) inhibition assay for compound 4b	38
55	Figure S55. Epidermal growth factor receptor (EGFR) inhibition assay for compound 4a	39
56	Figure S56. Casein kinase I (CK1) inhibition assay	40
57	Figure S57. P-gp substrate assay.	41
58	Figure S58. IC ₅₀ of compounds 4a (IC ₂₆₁), 4b , 4d , 4e , 6e and 6f against COLO-205 using SRB assay.	42
59	Figure S59. Cytotoxicity assay and IC ₅₀ of compounds 4a , 4b and 5-FU against A549 cell line	43
60	Cell culture protocol	44
61	Figure S60. Raw data of cytotoxicity assay and IC ₅₀ of compounds 4a , 4b and 5-FU against A549 cell line.	46
62	Figure S61. Elemental analysis data.	47

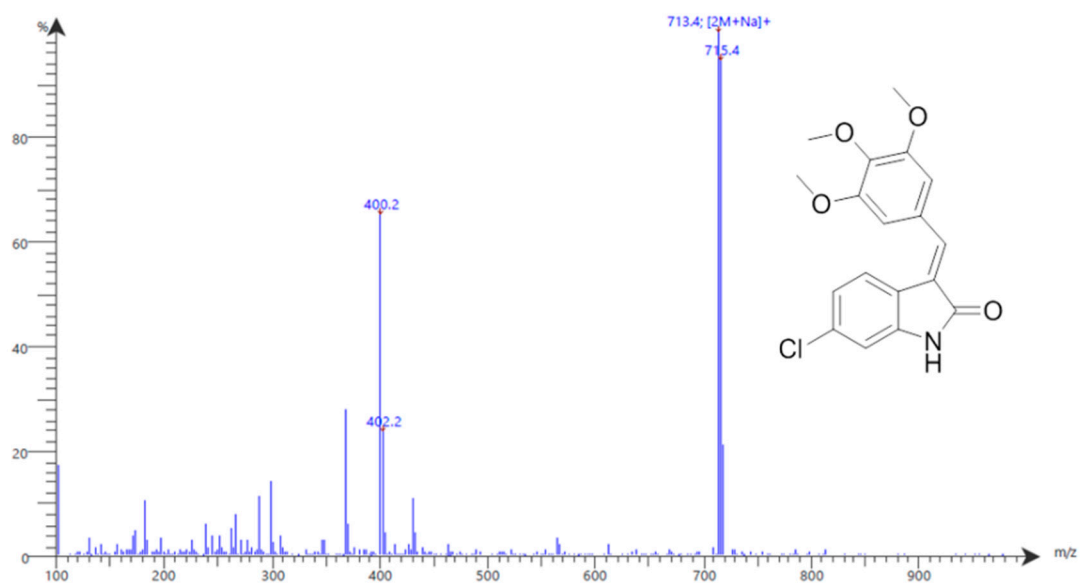


Figure S1. LC/MS spectrum of compound **3b**.

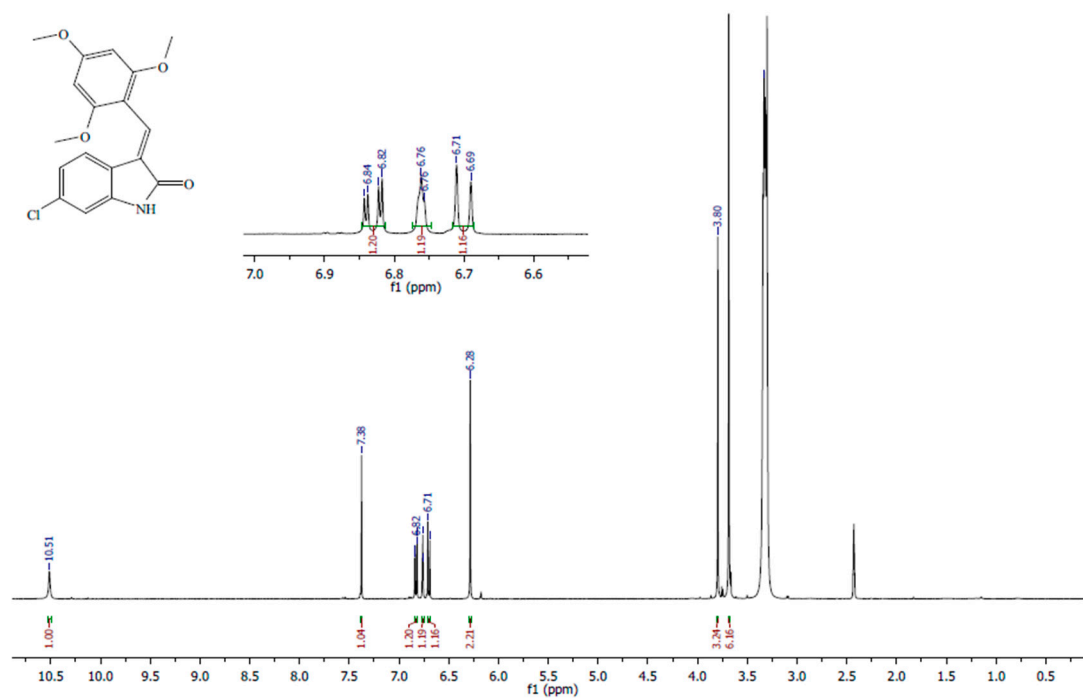


Figure S2. ¹H-NMR spectrum of compound **4b** in DMSO-*d*₆ at 400MHz.

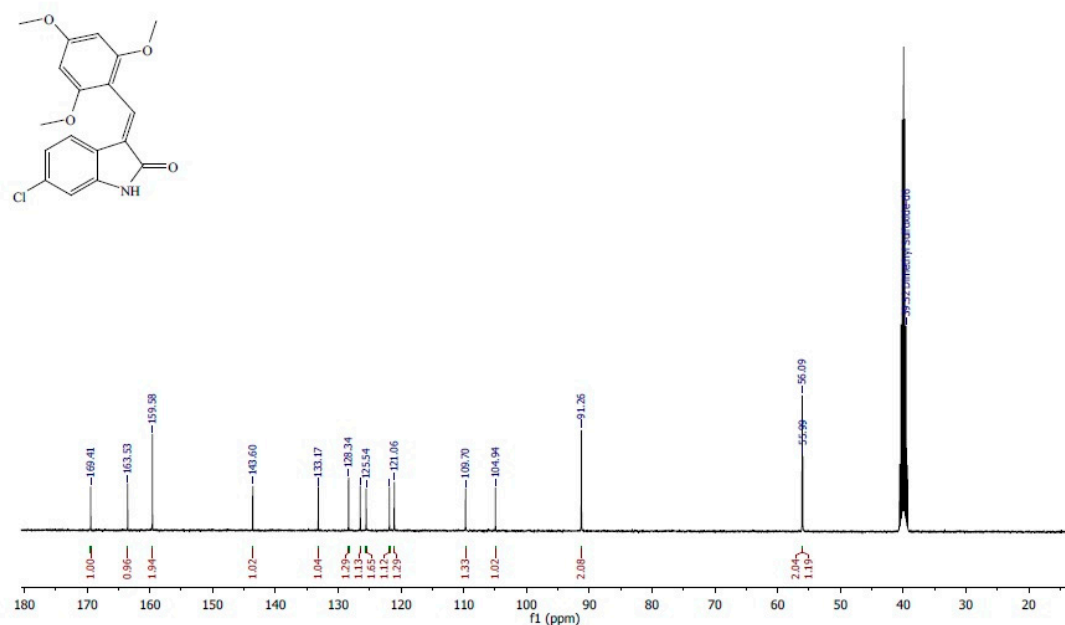


Figure S3. ¹³C-NMR spectrum of compound **4b** in DMSO-*d*₆ at 100MHz.

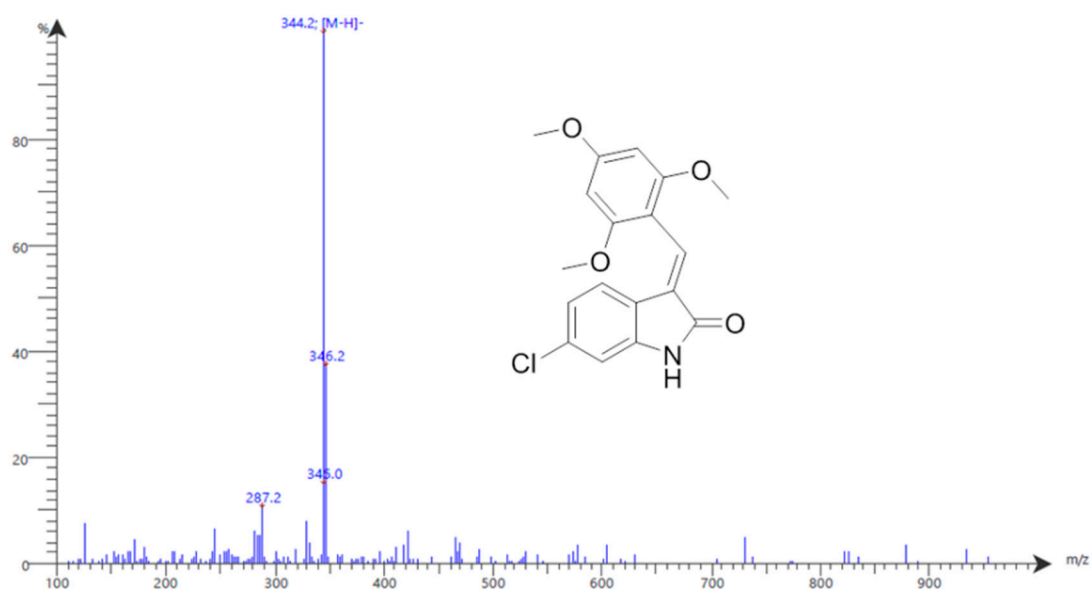


Figure S4. LC/MS spectrum of compound **4b**.

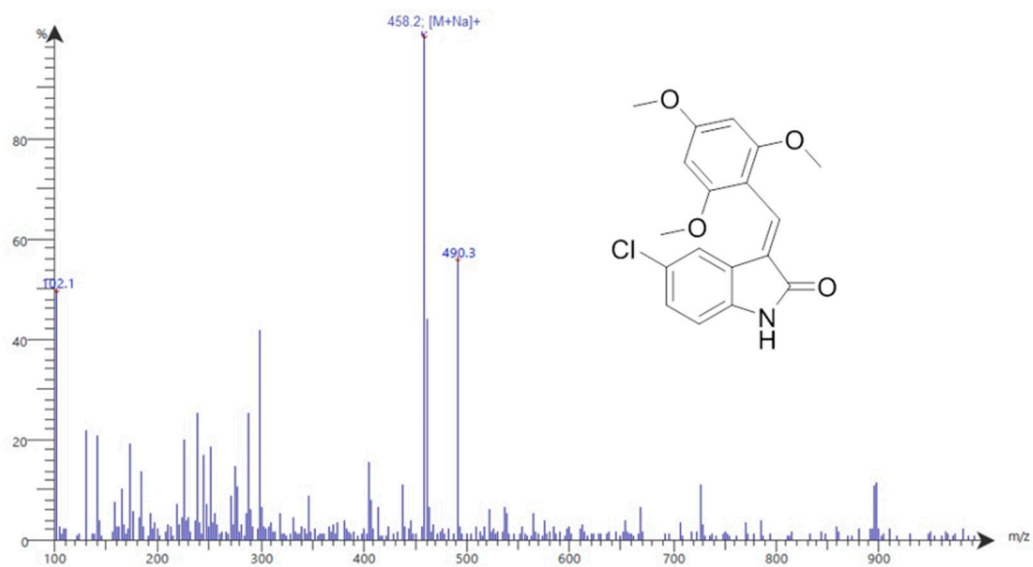


Figure S5. LC/MS spectrum of compound **4c**.

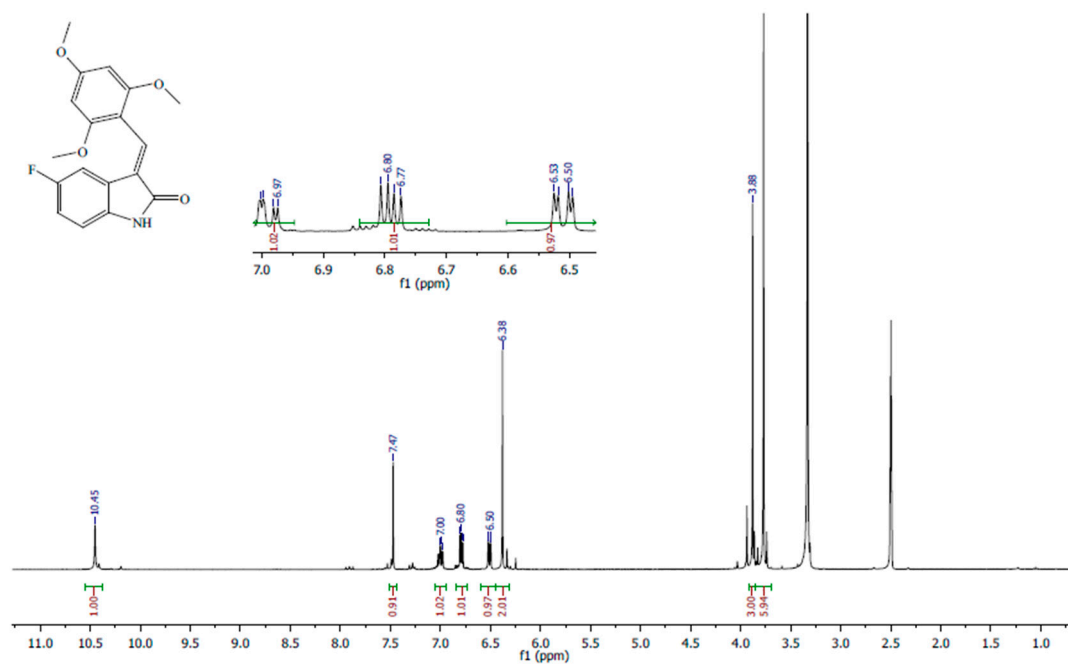


Figure S6. ^1H -NMR spectrum of compound **4d** in $\text{DMSO}-d_6$ at 500MHz.

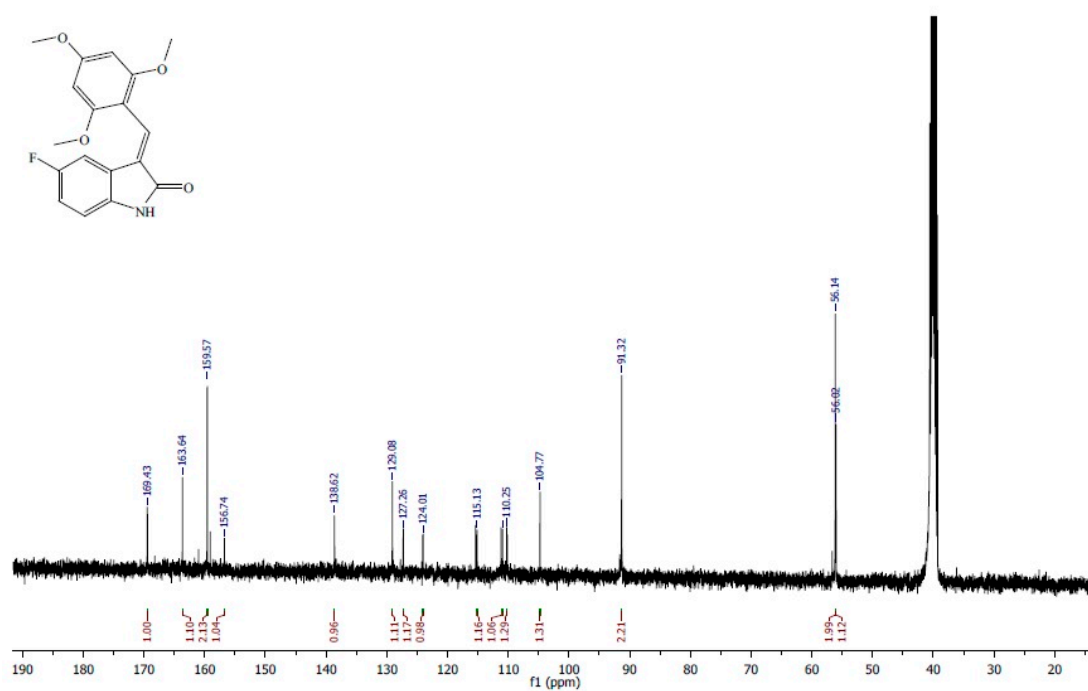


Figure S7. ^{13}C -NMR spectrum of compound **4d** in $\text{DMSO}-d_6$ at 100MHz.

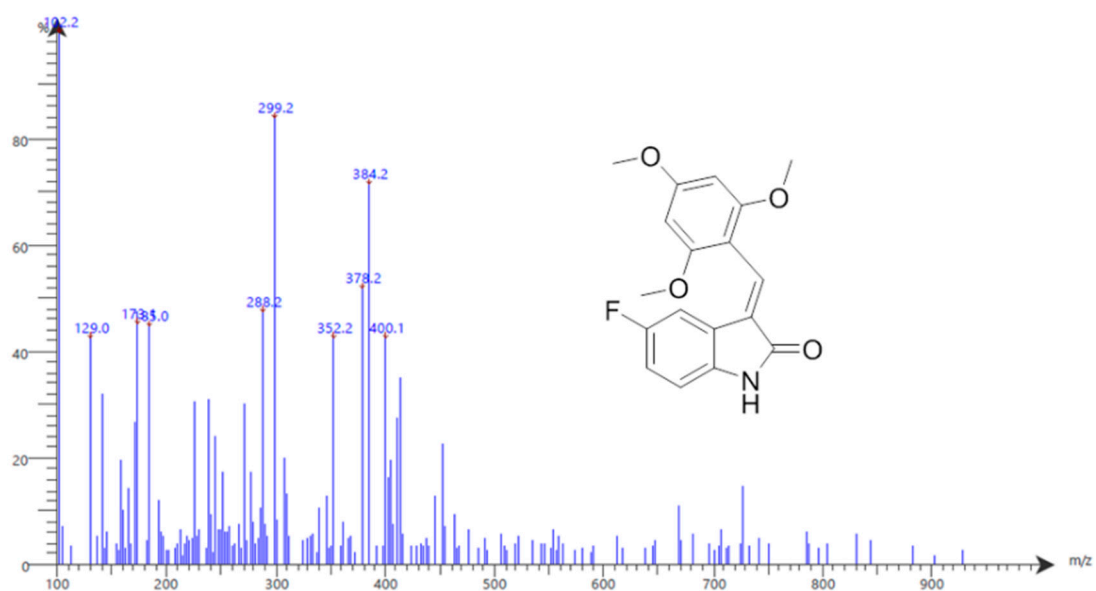


Figure S8. LC/MS spectrum of compound **4d**.

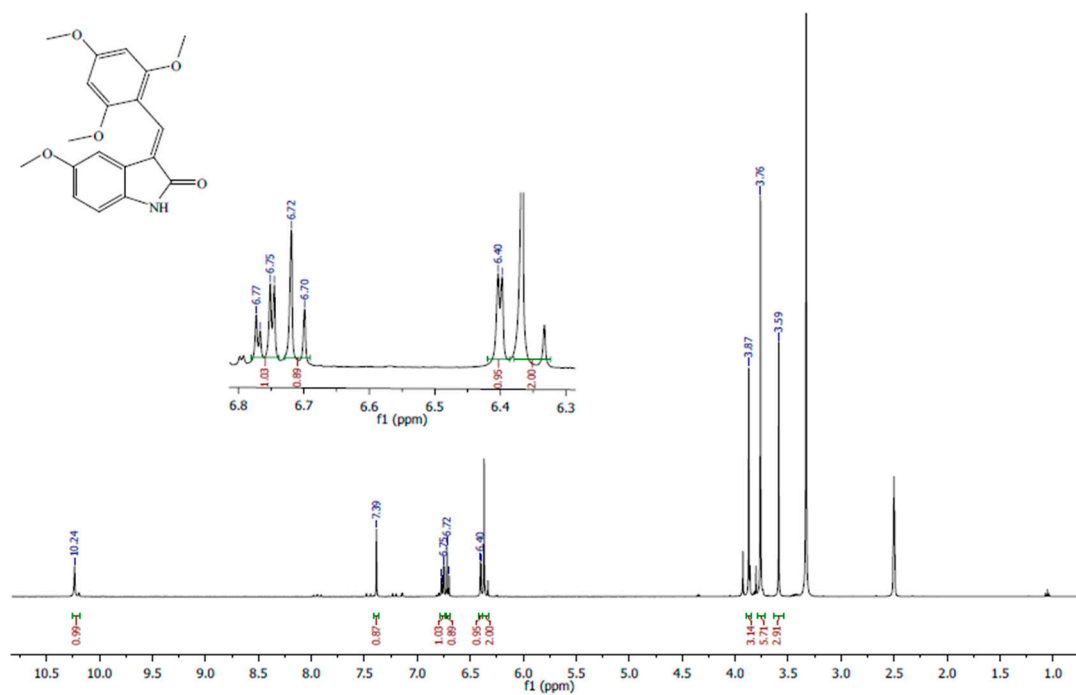


Figure S9. ¹H-NMR spectrum of compound **4e** in DMSO-*d*₆ at 400MHz.

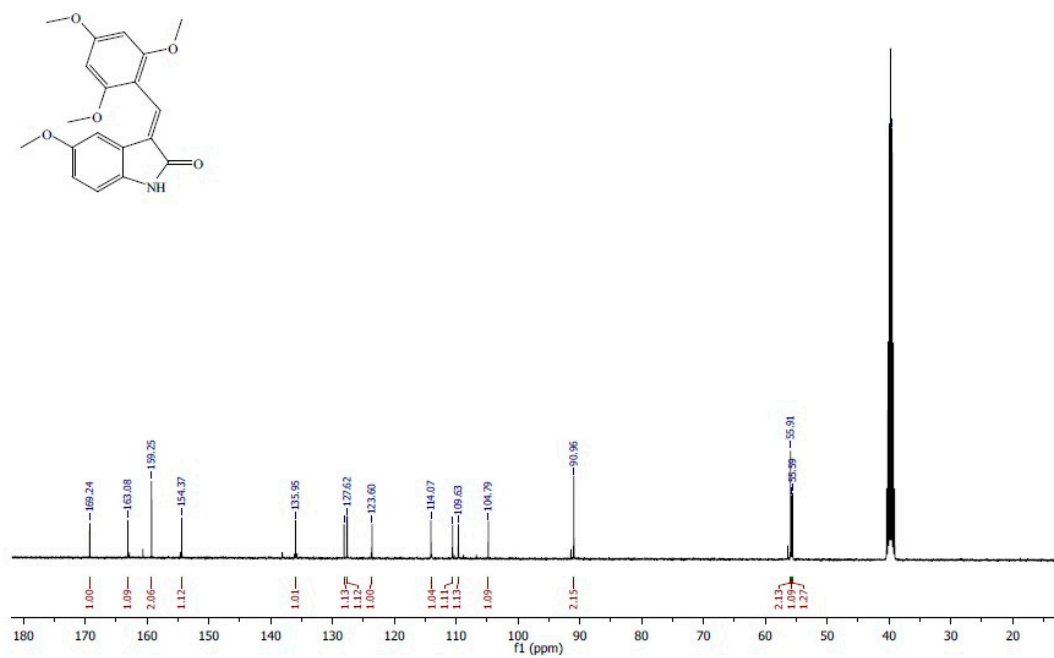


Figure S10. ¹³C-NMR spectrum of compound **4e** in DMSO-*d*₆ at 100MHz.

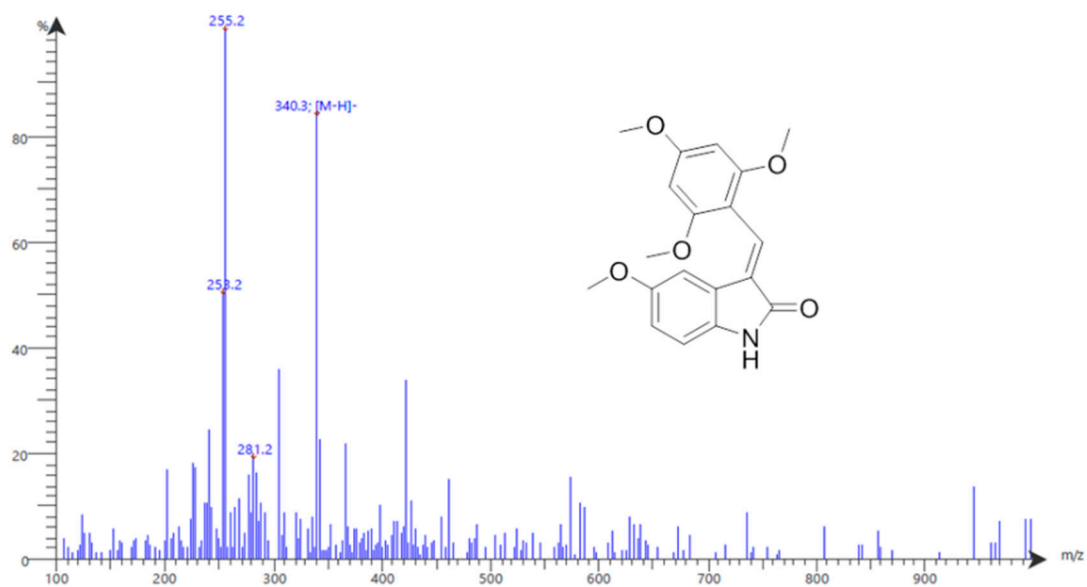


Figure S11. LC/MS spectrum of compound **4e**.

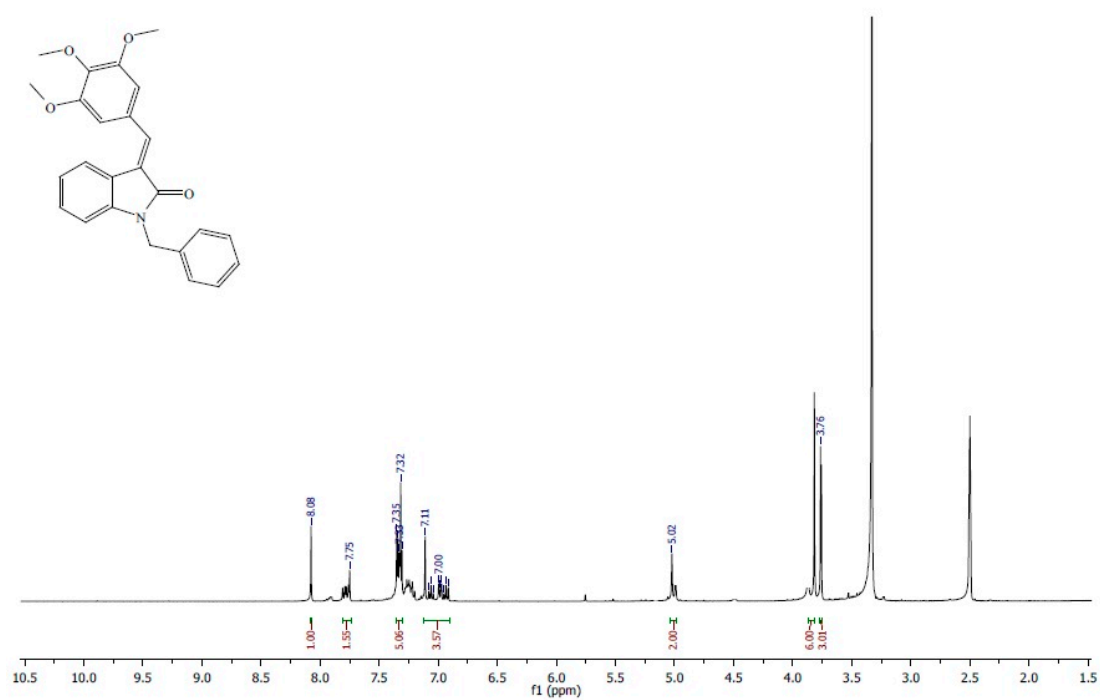


Figure S12. ¹H-NMR spectrum of compound **6a** in DMSO-*d*₆ at 400MHz.

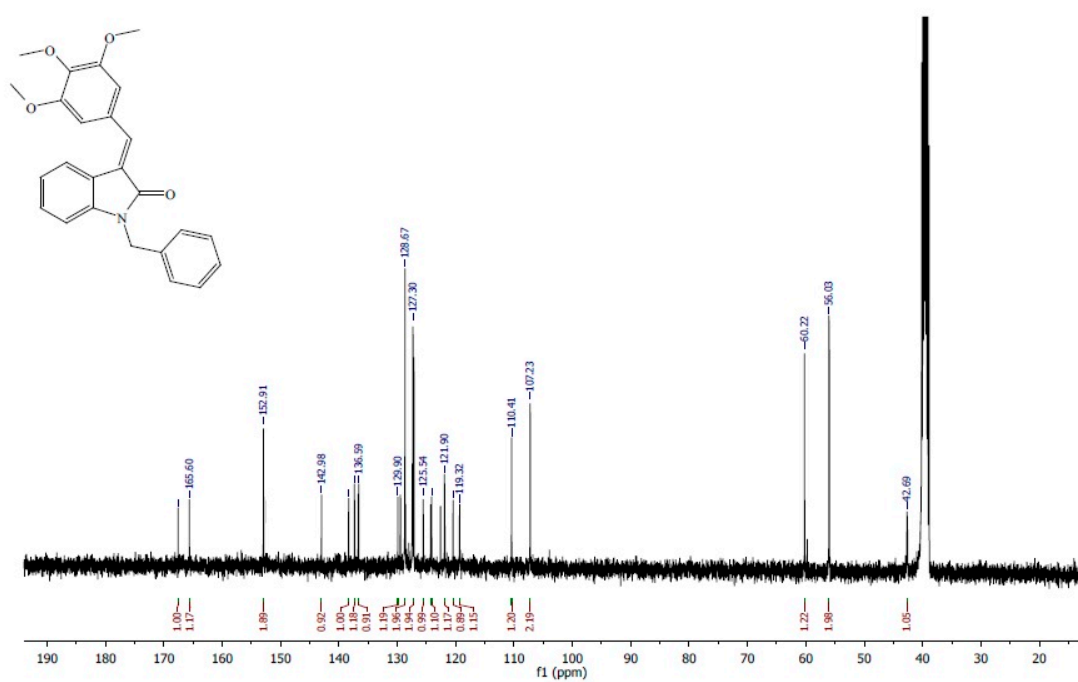


Figure S13. ¹³C-NMR spectrum of compound **6a** in DMSO-*d*₆ at 100MHz.

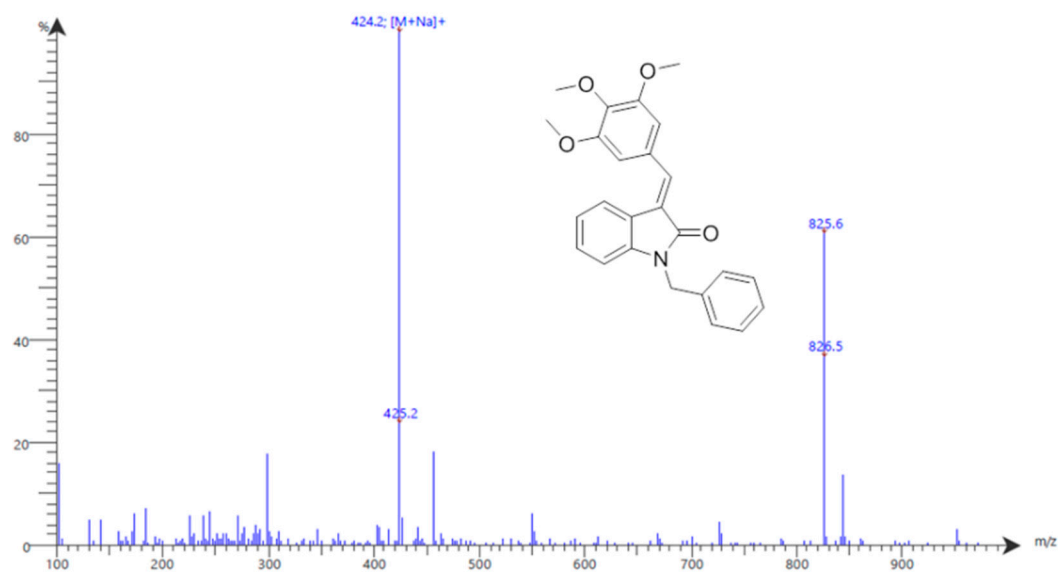


Figure S14. LC/MS spectrum of compound **6a**.

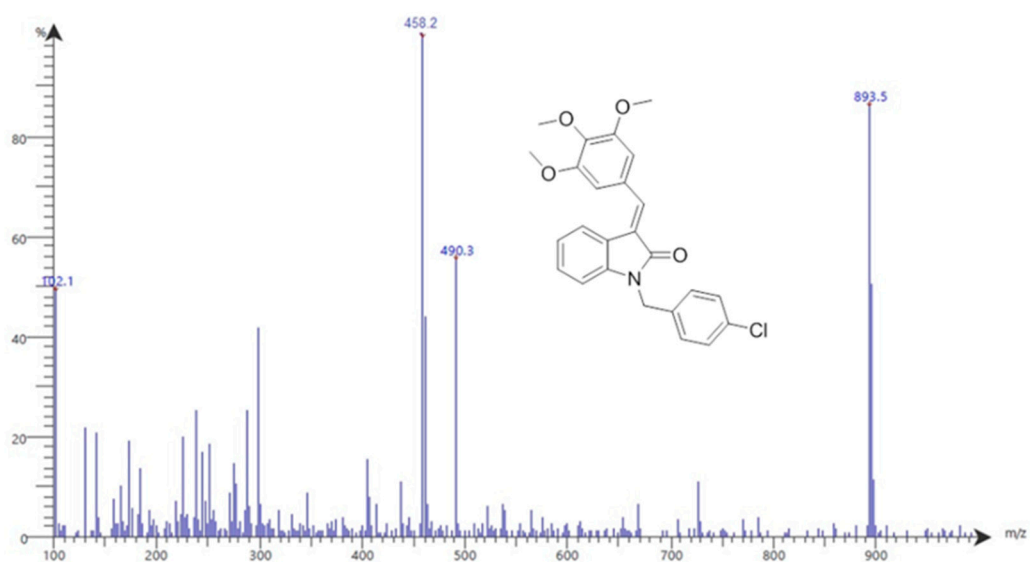


Figure S15. LC/MS spectrum of compound **6b**.

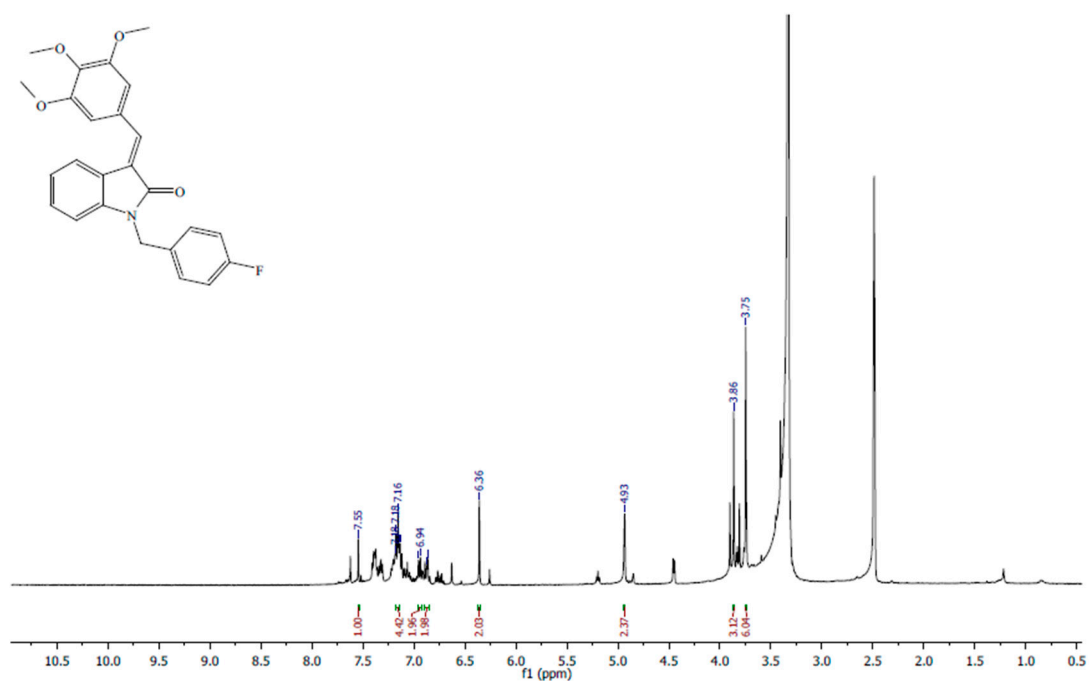


Figure S16. ¹H-NMR spectrum of compound **6c** in DMSO-*d*₆ at 400MHz.

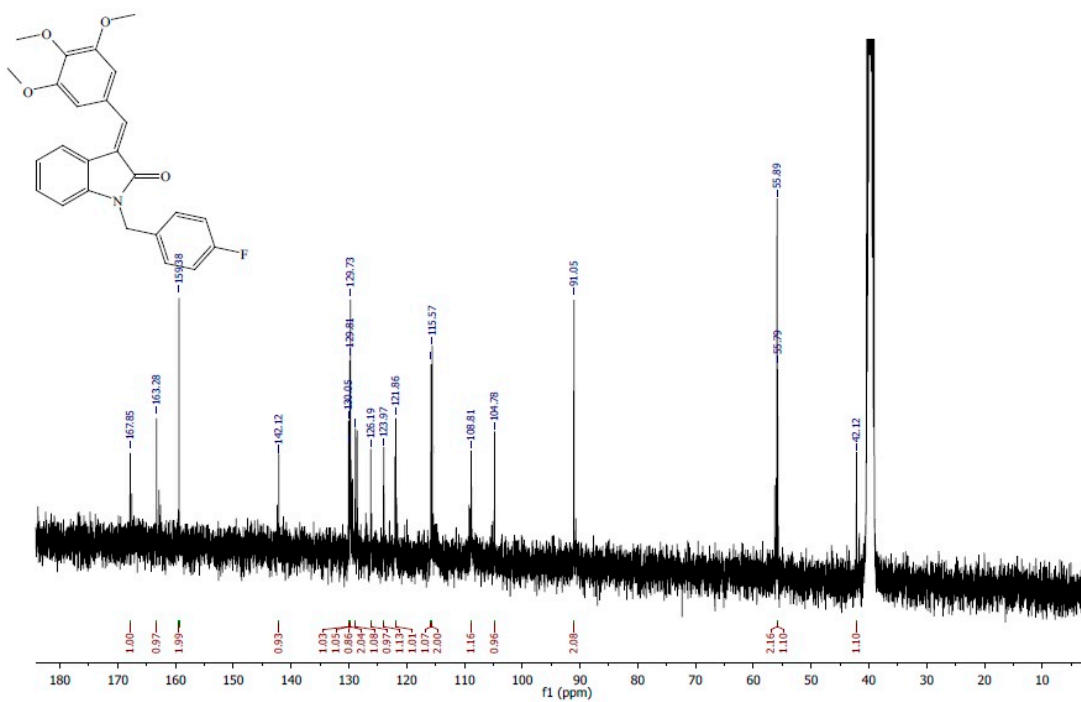


Figure S17. ¹³C-NMR spectrum of compound **6c** in DMSO-*d*₆ at 100MHz.

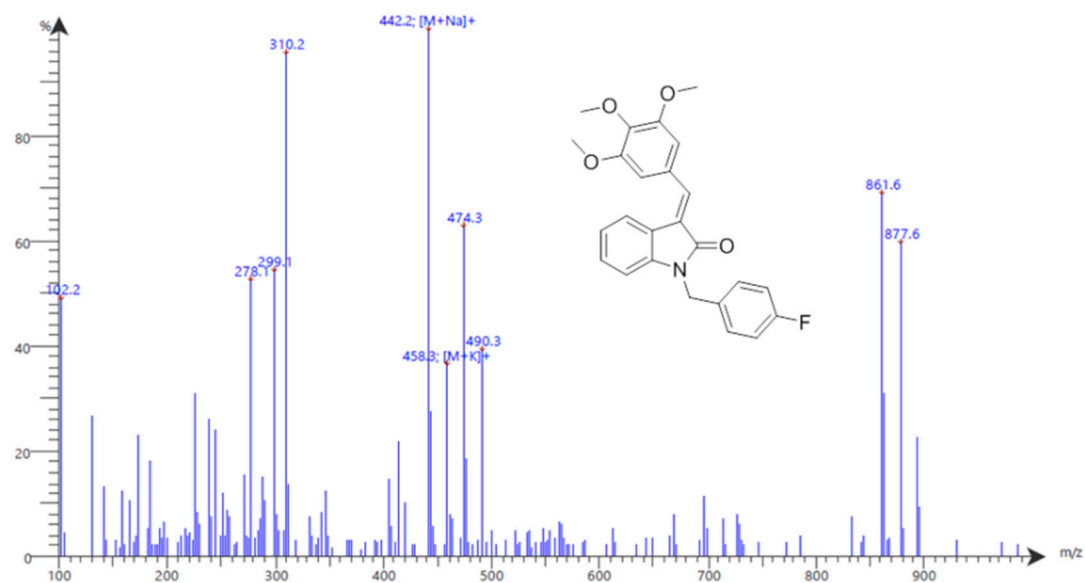


Figure S18. LC/MS spectrum of compound **6c**.

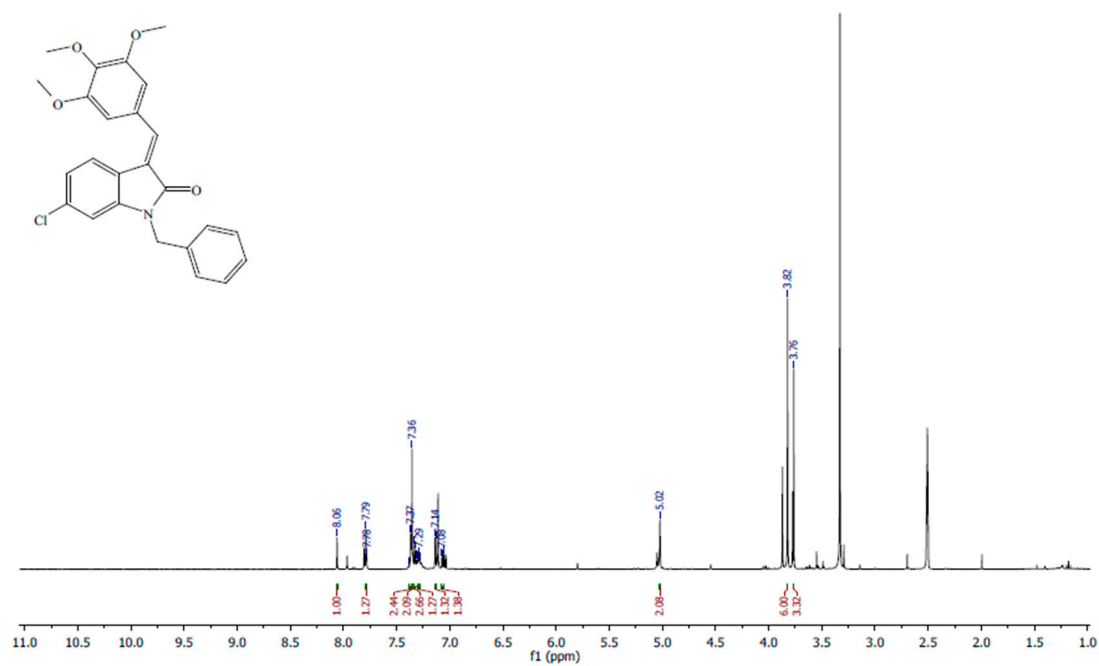


Figure S19. ¹H-NMR spectrum of compound **6d** in DMSO-*d*₆ at 400MHz.

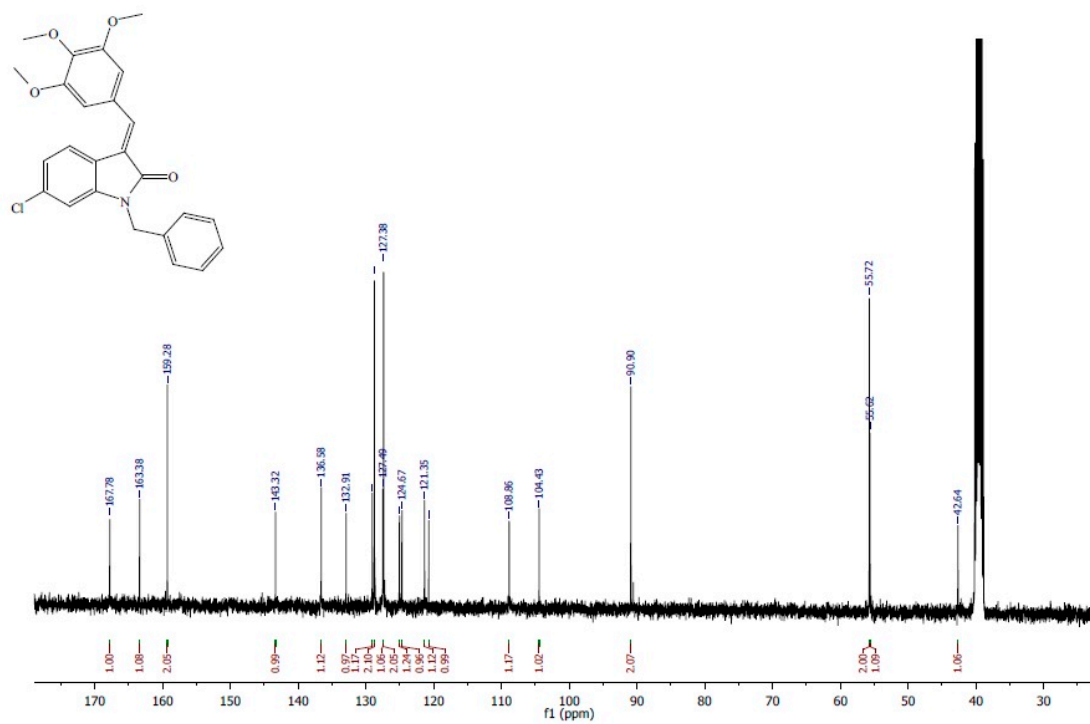


Figure S20. ¹³C-NMR spectrum of compound **6d** in DMSO-*d*₆ at 100MHz.

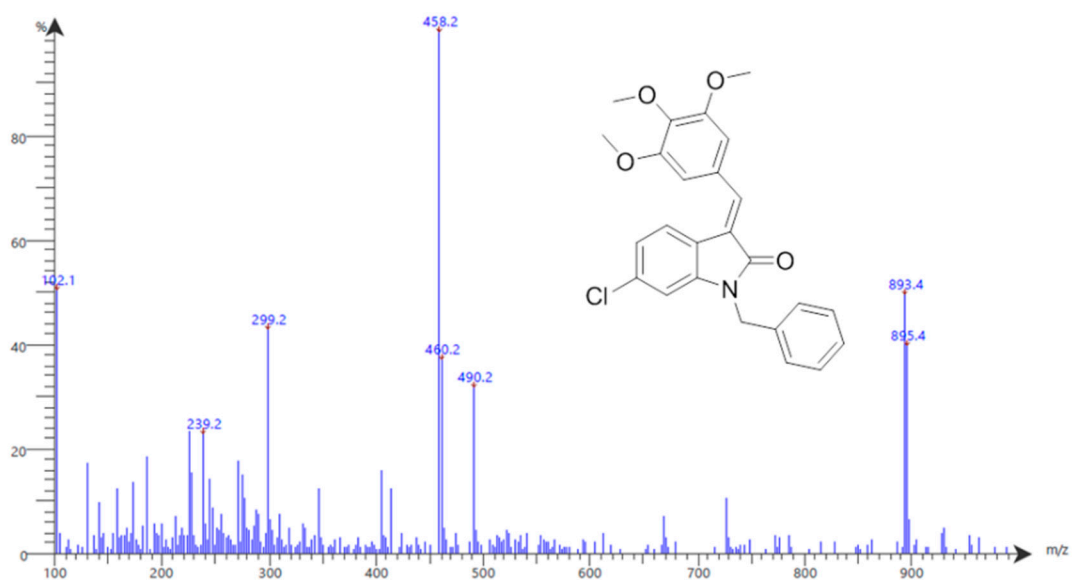


Figure S21. LC/MS spectrum of compound **6d**.

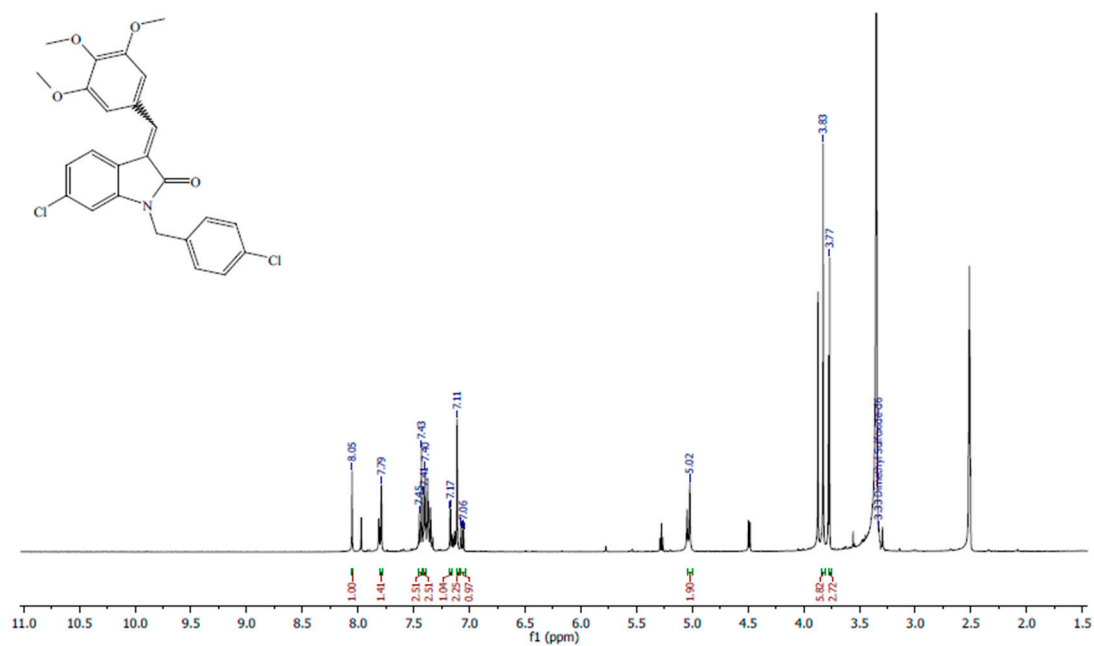


Figure S22. ¹H-NMR spectrum of compound **6e** in DMSO-*d*₆ at 400MHz.

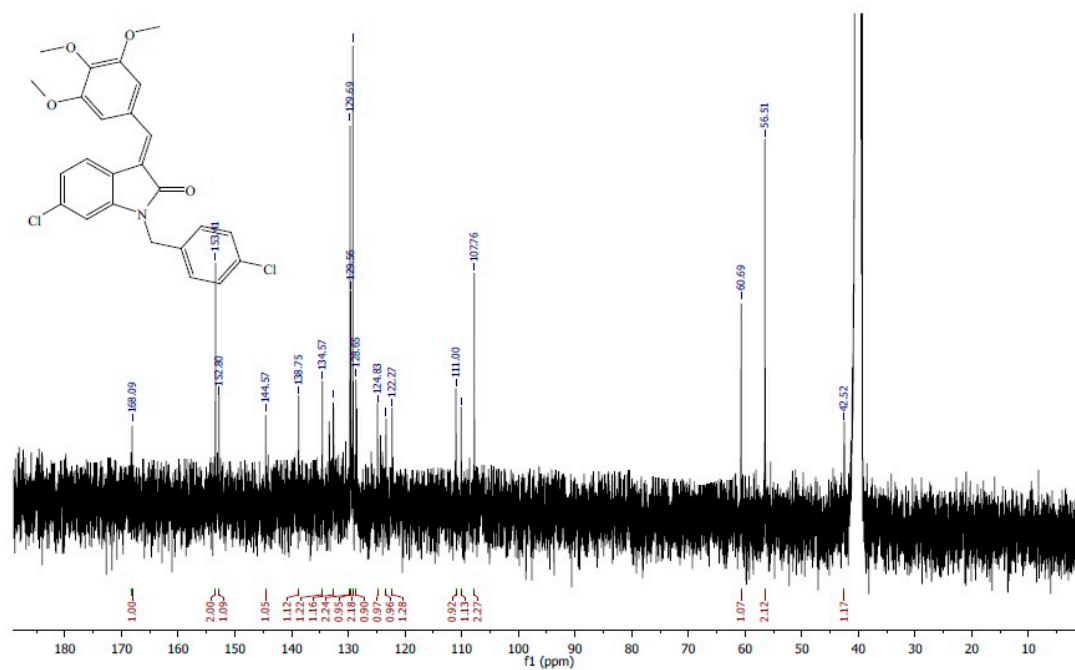


Figure S23. ¹³C-NMR spectrum of compound **6e** in DMSO-*d*₆ at 100MHz.

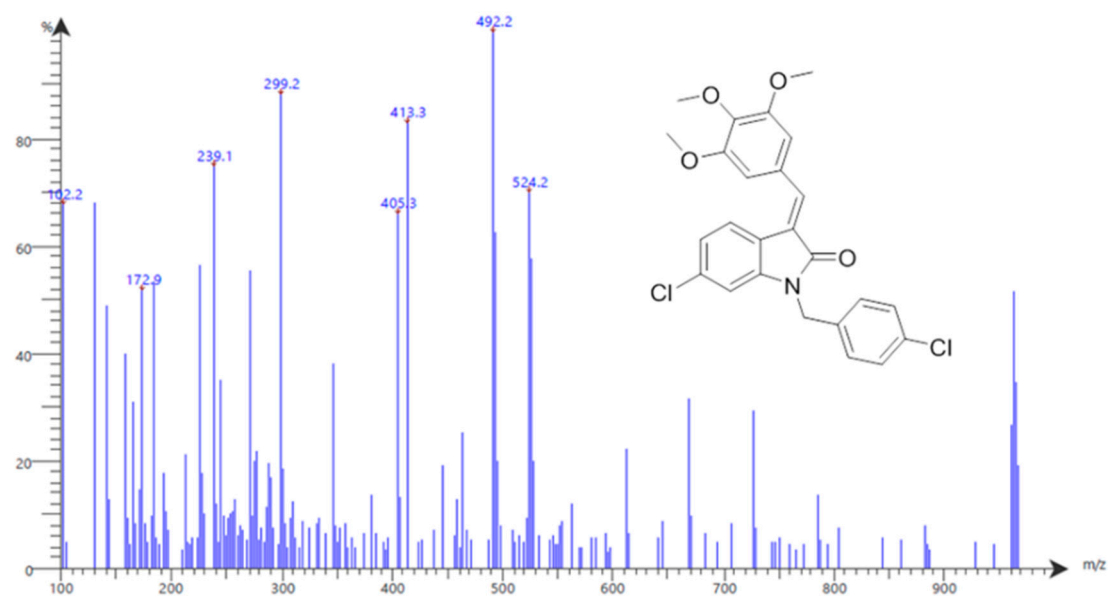


Figure S24. LC/MS spectrum of compound **6e**.

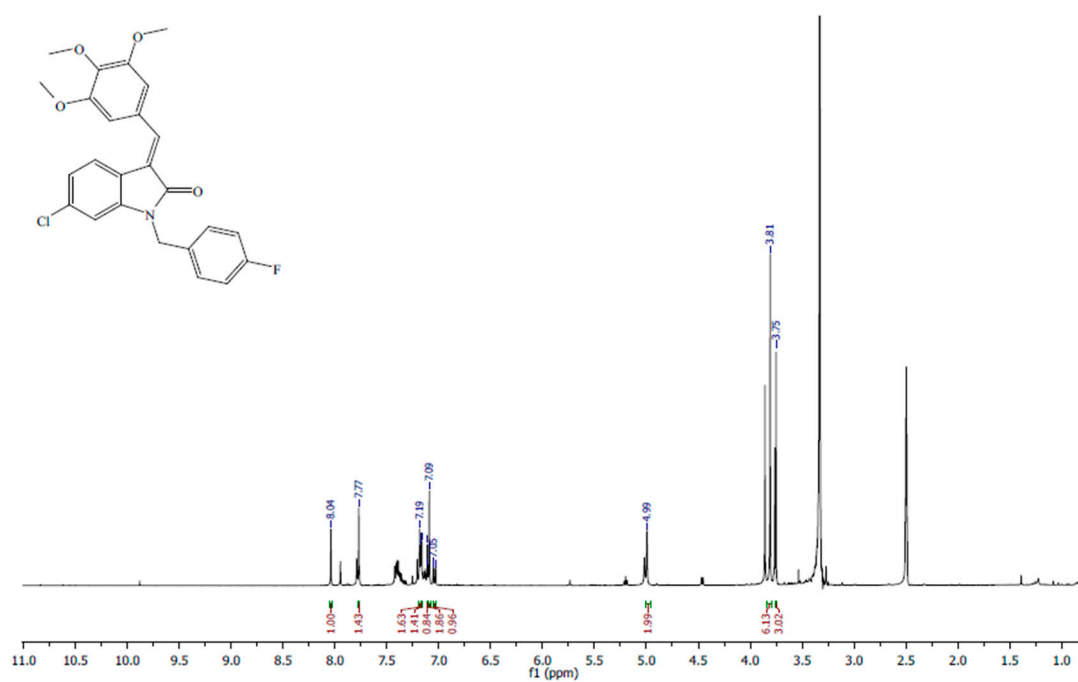


Figure S25. ^1H -NMR spectrum of compound **6f** in $\text{DMSO-}d_6$ at 400MHz.

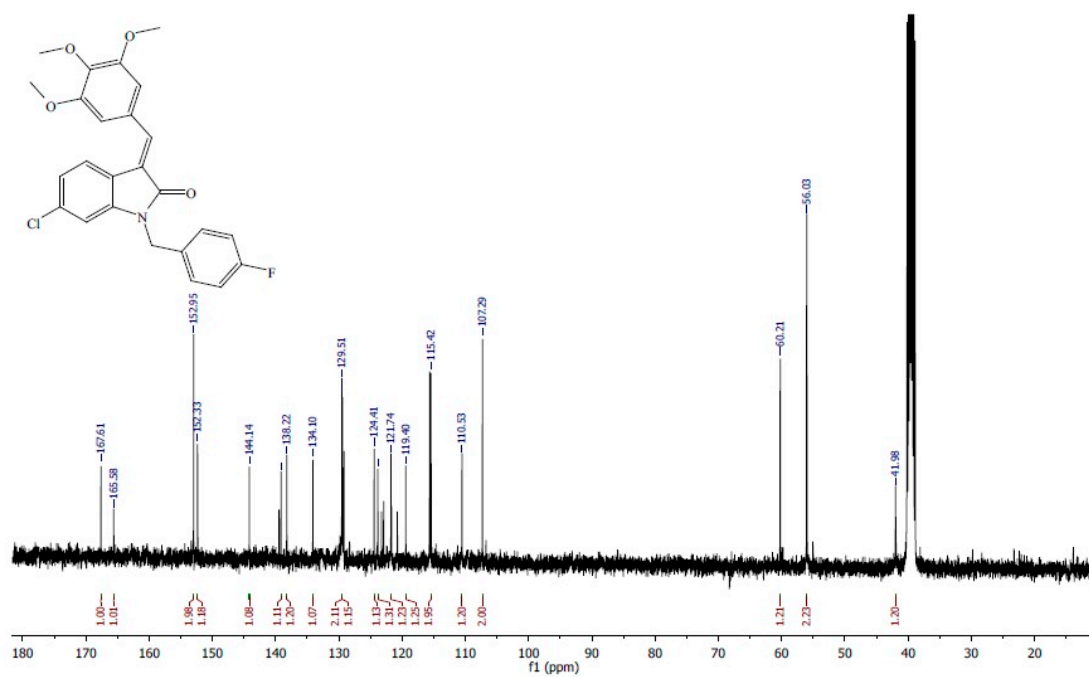


Figure S26. ^{13}C -NMR spectrum of compound **6f** in $\text{DMSO-}d_6$ at 100MHz.

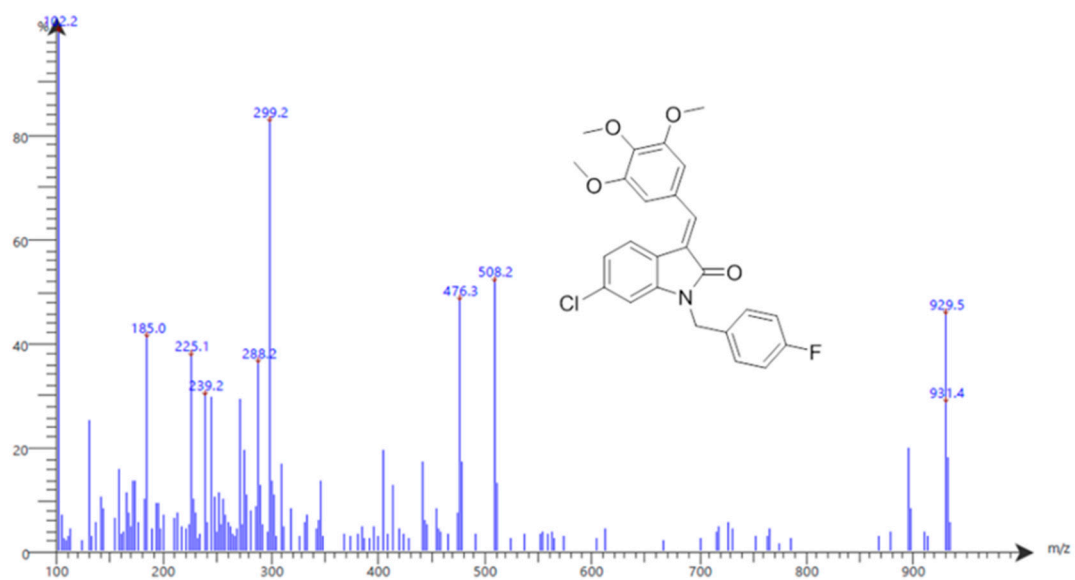


Figure S27. LC/MS spectrum of compound **6f**.

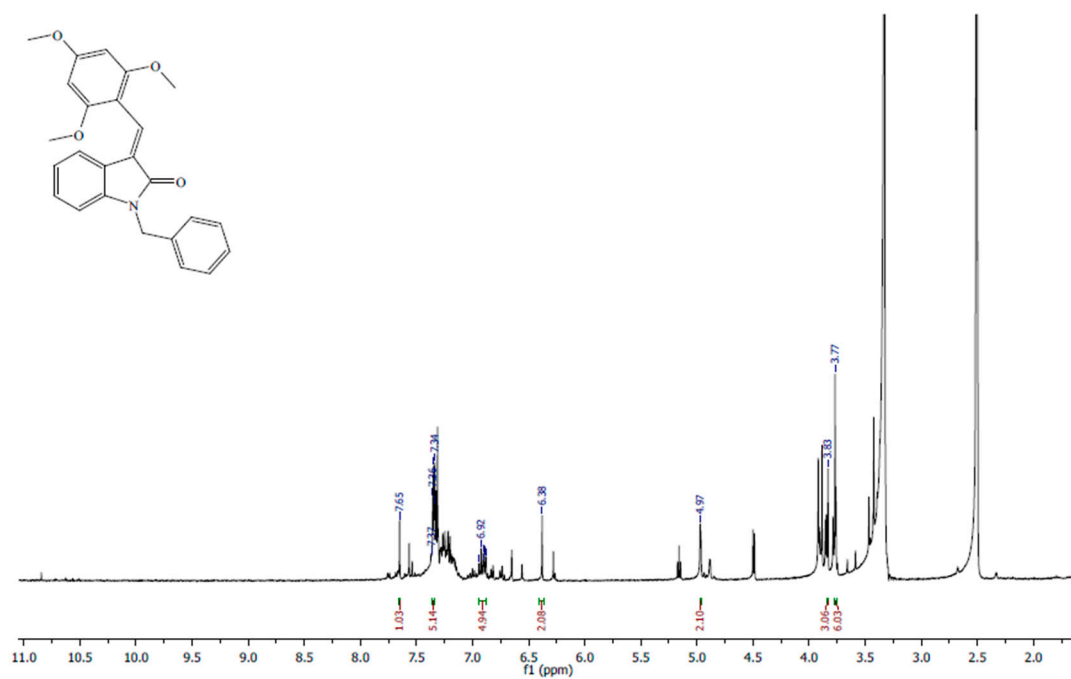


Figure S28. ¹H-NMR spectrum of compound **7a** in DMSO-*d*₆ at 400MHz.

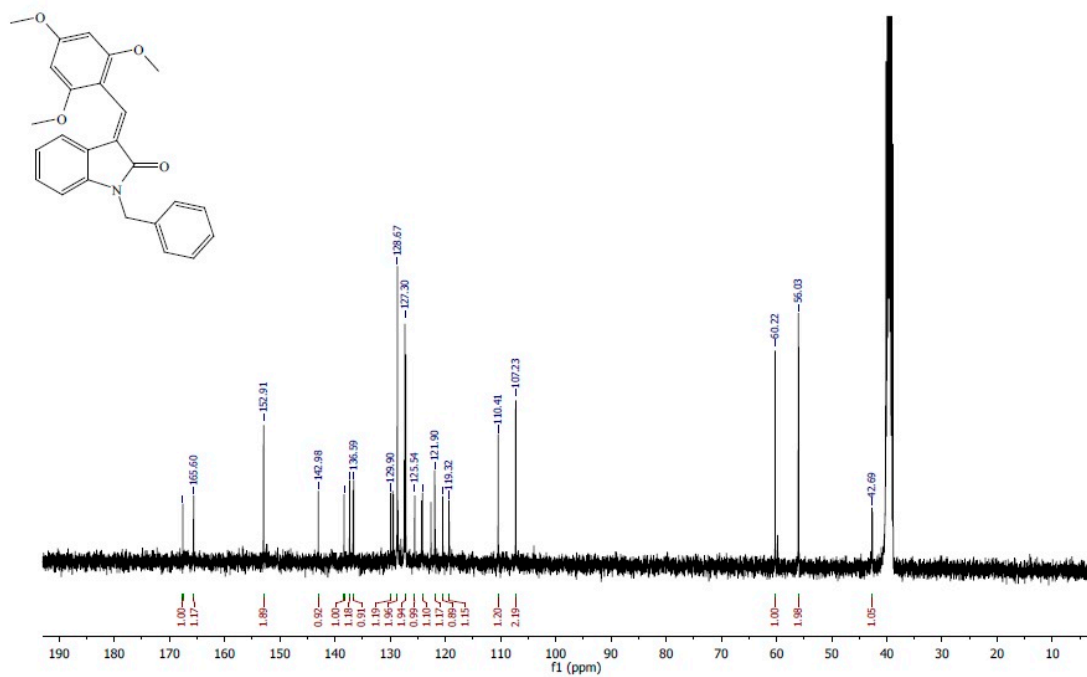


Figure S29. ¹³C-NMR spectrum of compound **7a** in DMSO-*d*₆ at 100MHz.

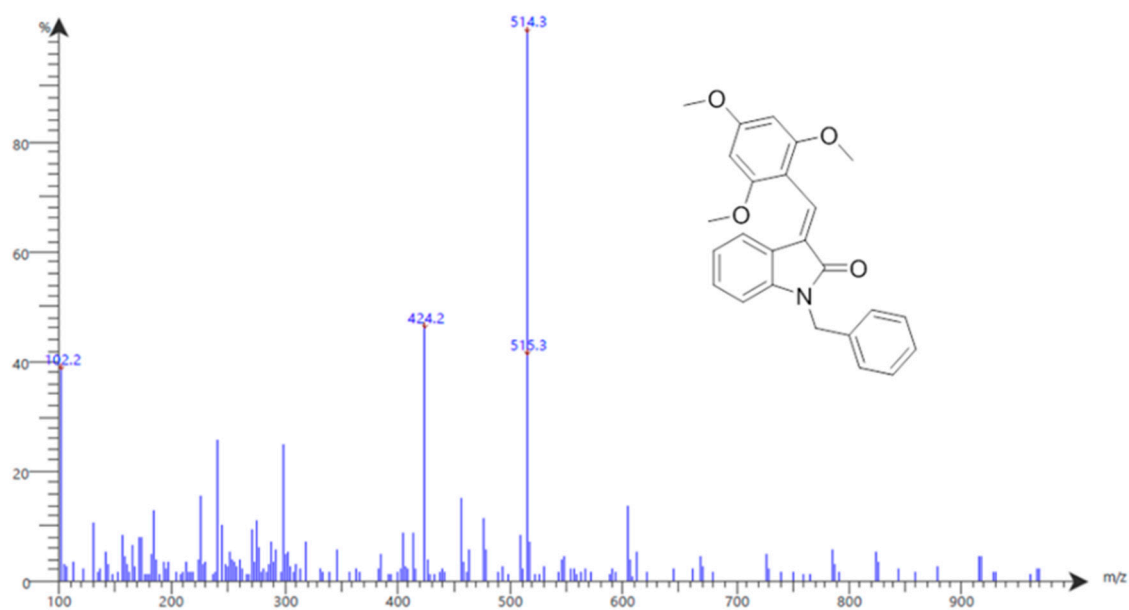


Figure S30. LC/MS spectrum of compound **7a**.

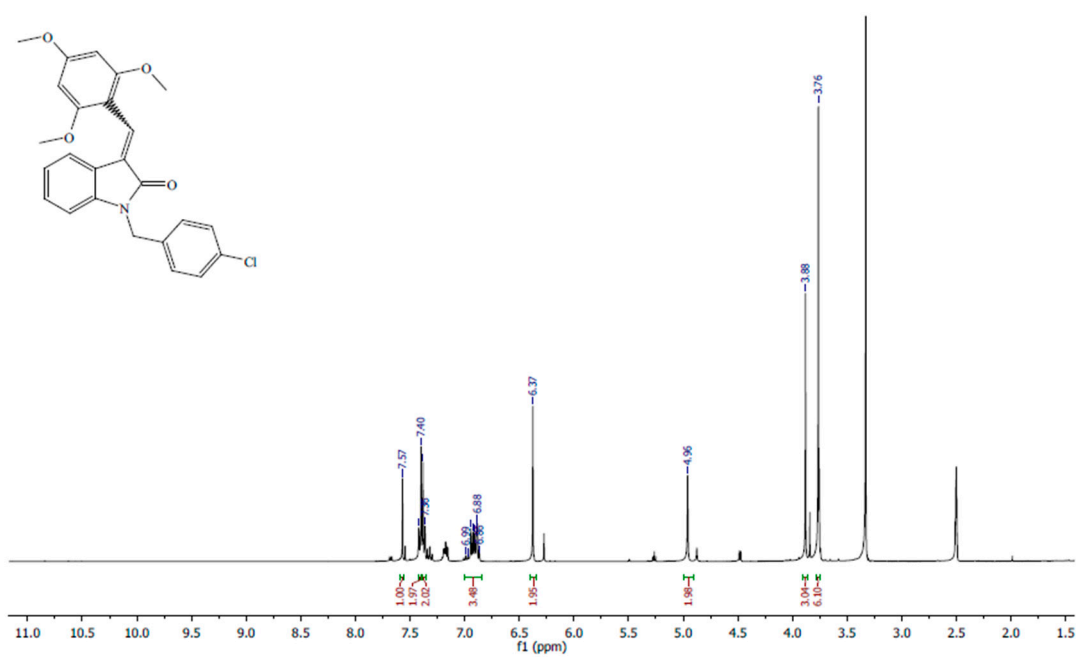


Figure S31. ^1H -NMR spectrum of compound **7b** in $\text{DMSO-}d_6$ at 400MHz.

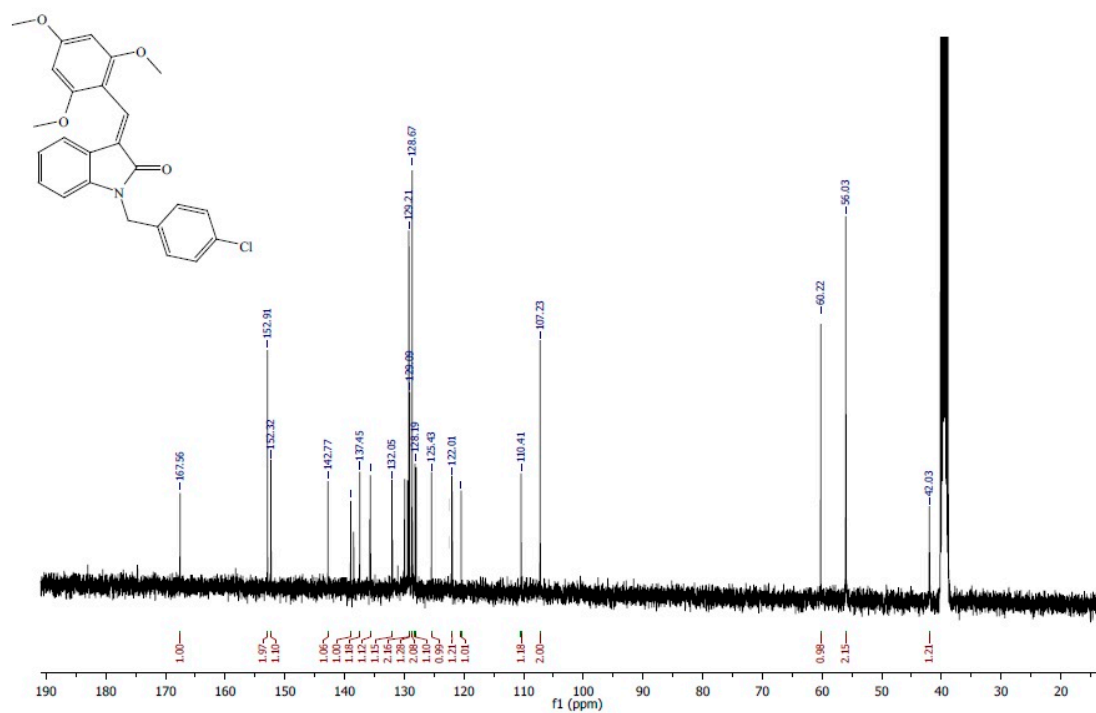


Figure S32. ¹³C-NMR spectrum of compound **7b** in DMSO-*d*₆ at 100MHz.

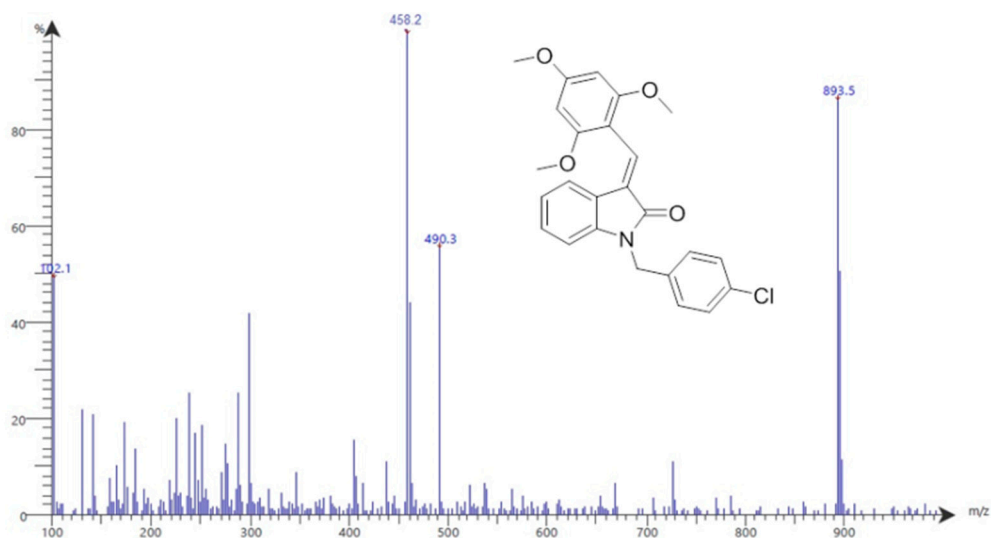


Figure S33. LC/MS spectrum of compound **7b**.

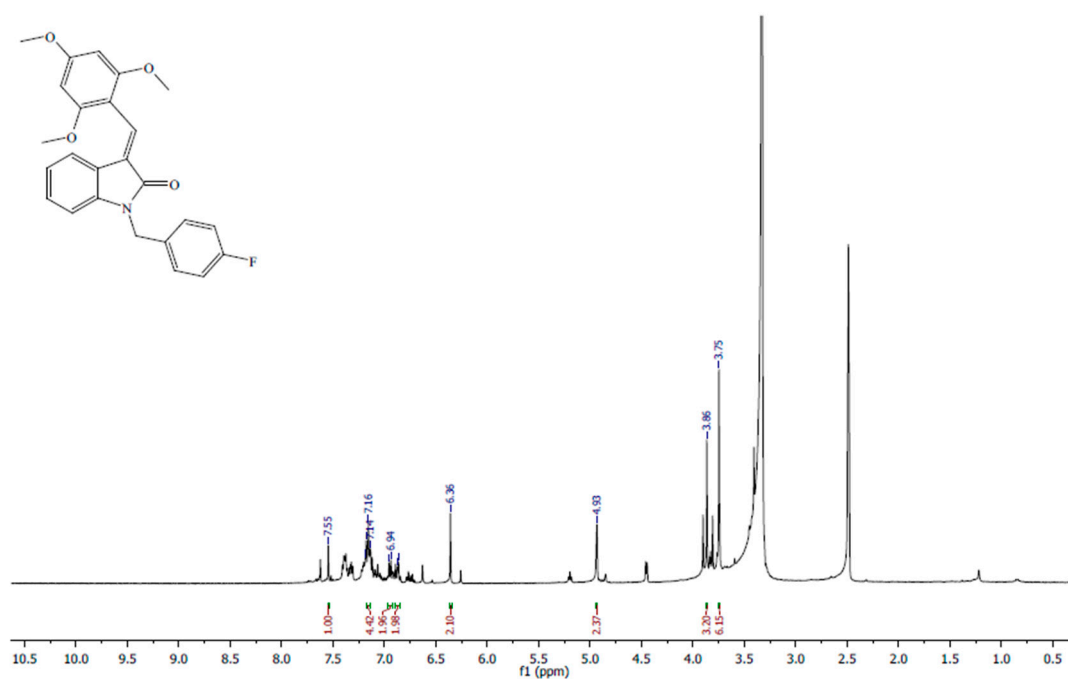


Figure S34. ¹H-NMR spectrum of compound 7c in DMSO-*d*₆ at 400MHz.

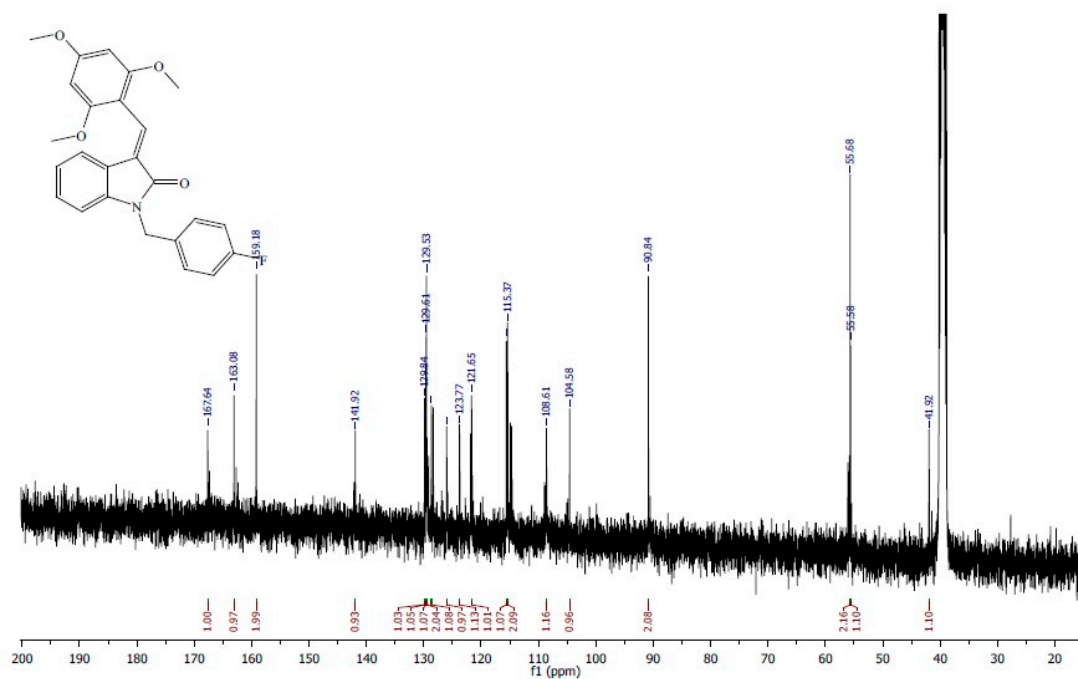


Figure S35. ¹³C-NMR spectrum of compound 7c in DMSO-*d*₆ at 100MHz.

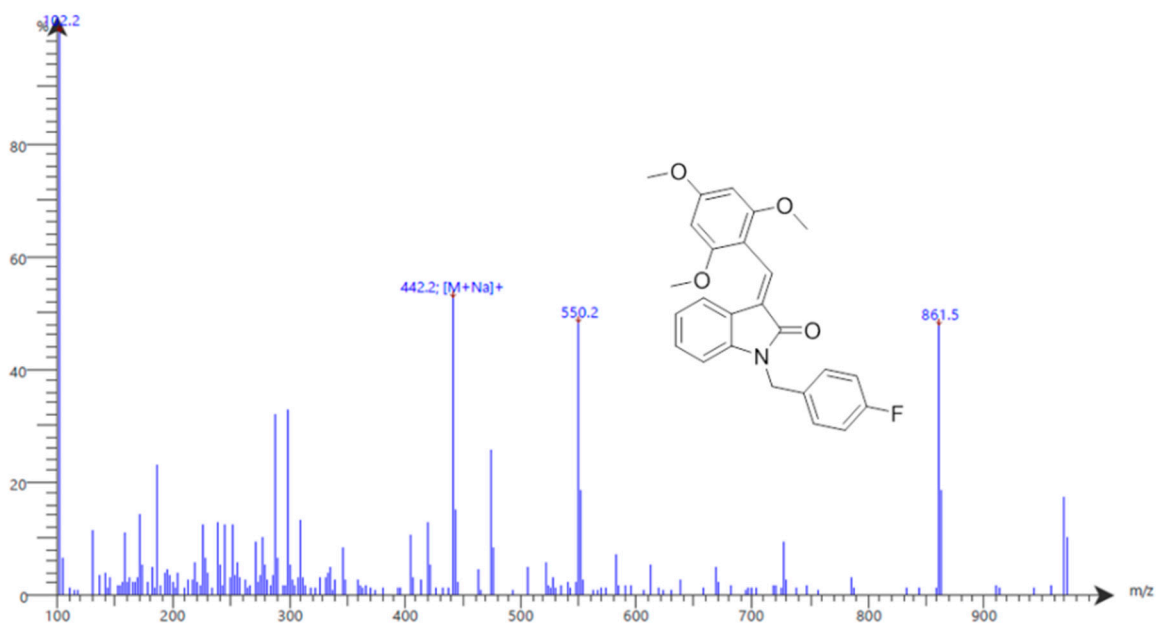


Figure S36. LC/MS spectrum of compound **7c**.

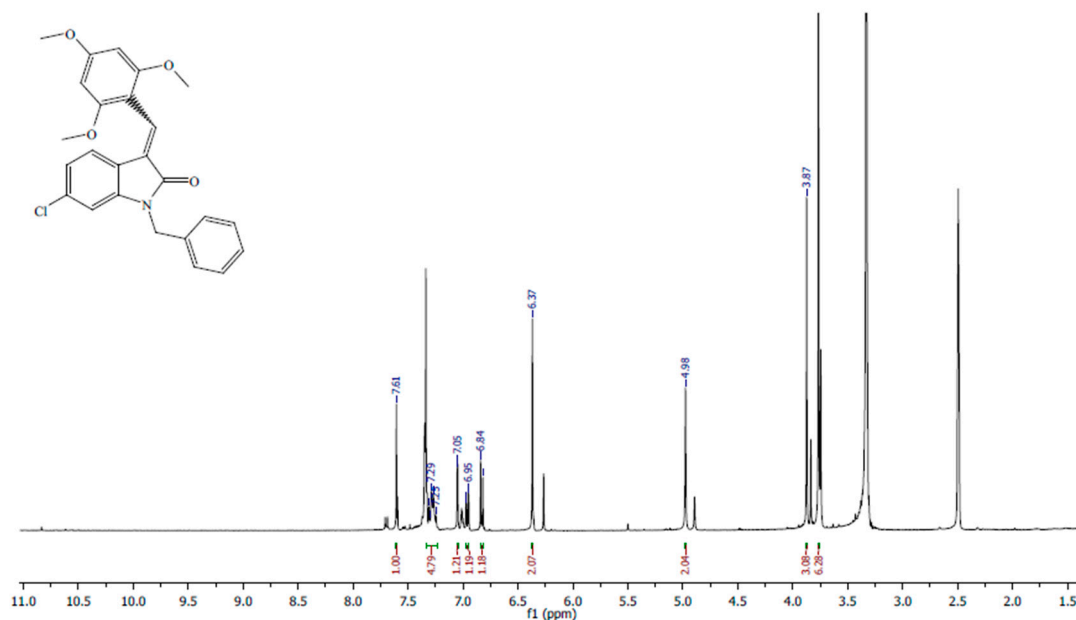


Figure S37. ^1H -NMR spectrum of compound **7d** in $\text{DMSO}-d_6$ at 400MHz.

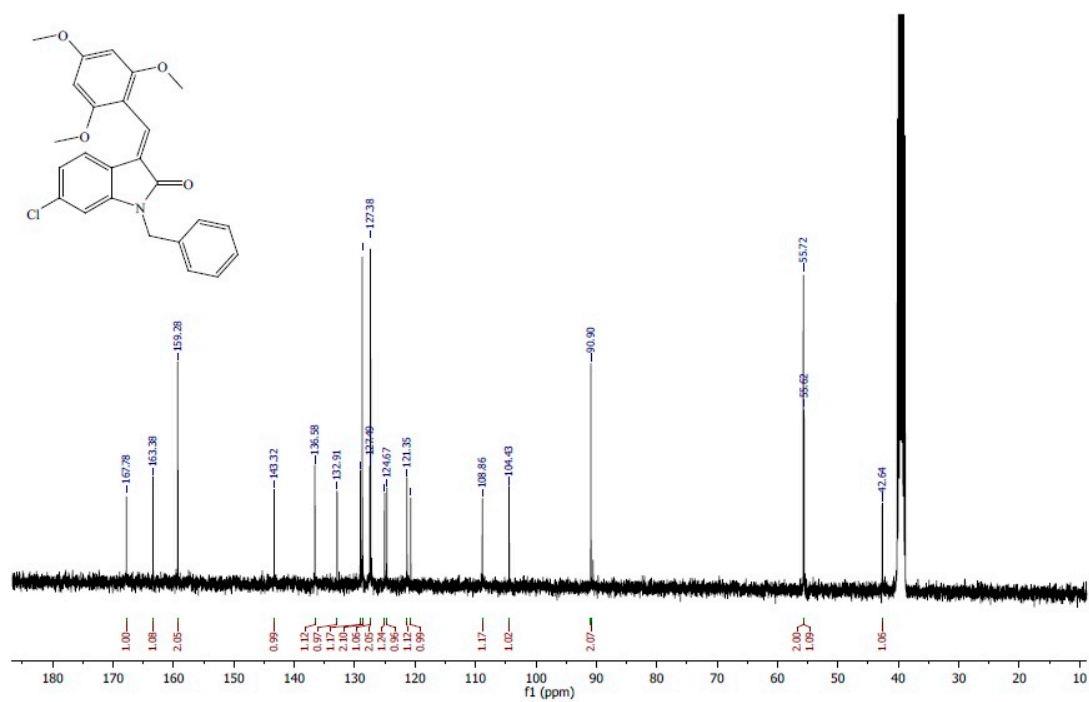


Figure S38. ¹³C-NMR spectrum of compound **7d** in DMSO-*d*₆ at 100MHz.

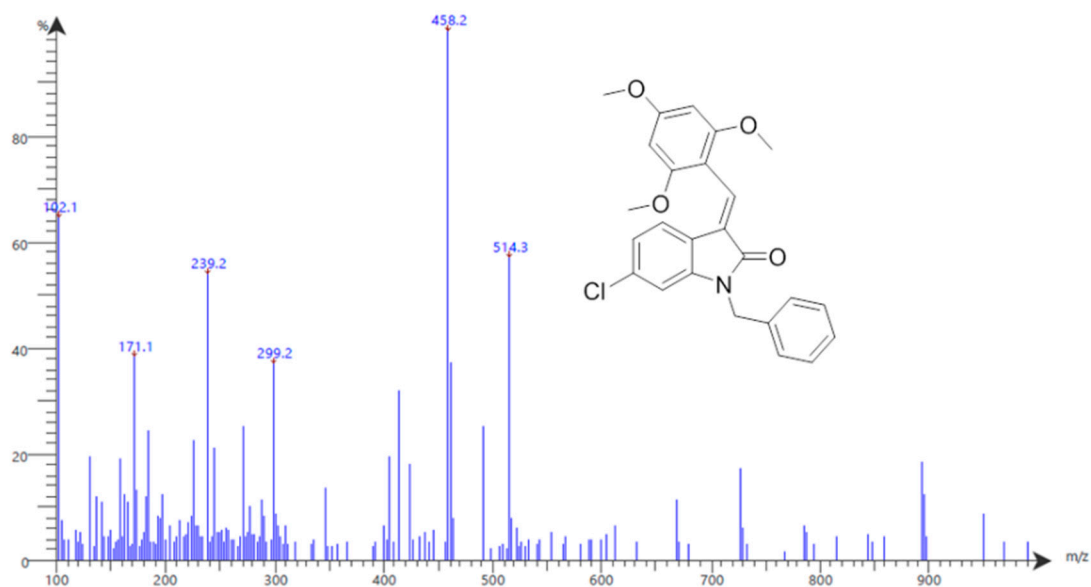


Figure S39. LC/MS spectrum of compound **7d**.

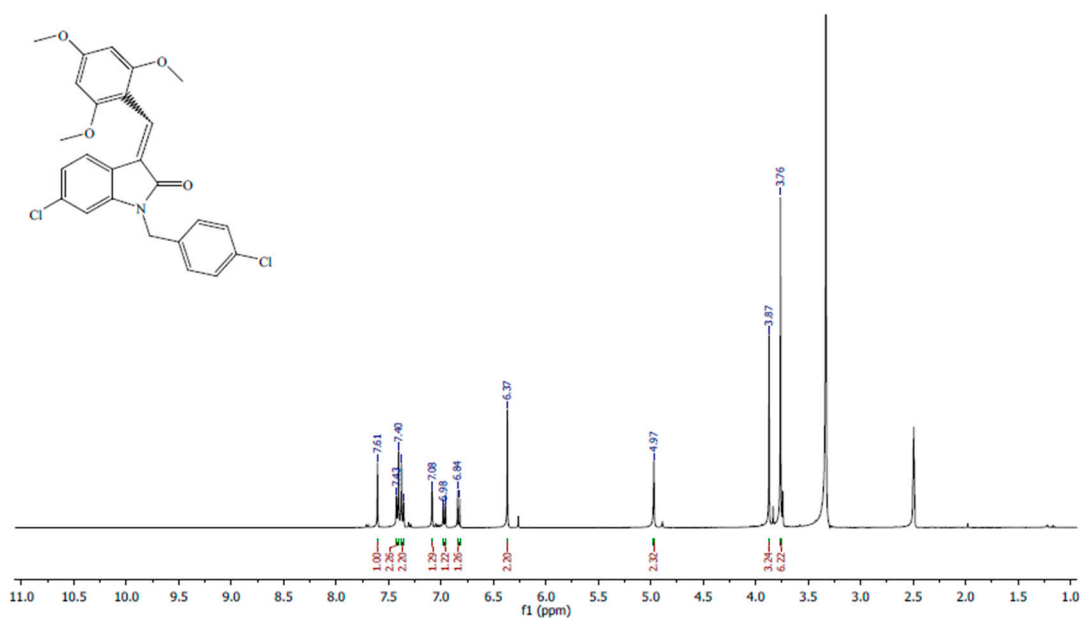


Figure S40. ^1H -NMR spectrum of compound **7e** in $\text{DMSO}-d_6$ at 400MHz.

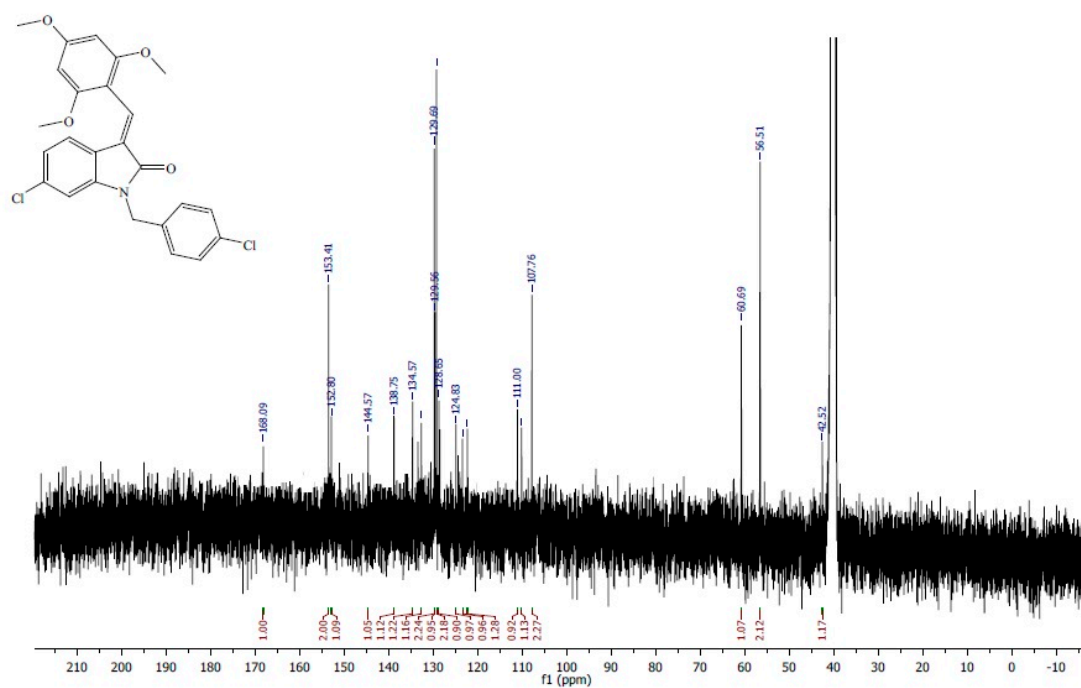


Figure S41. ^{13}C -NMR spectrum of compound **7e** in $\text{DMSO}-d_6$ at 100MHz.

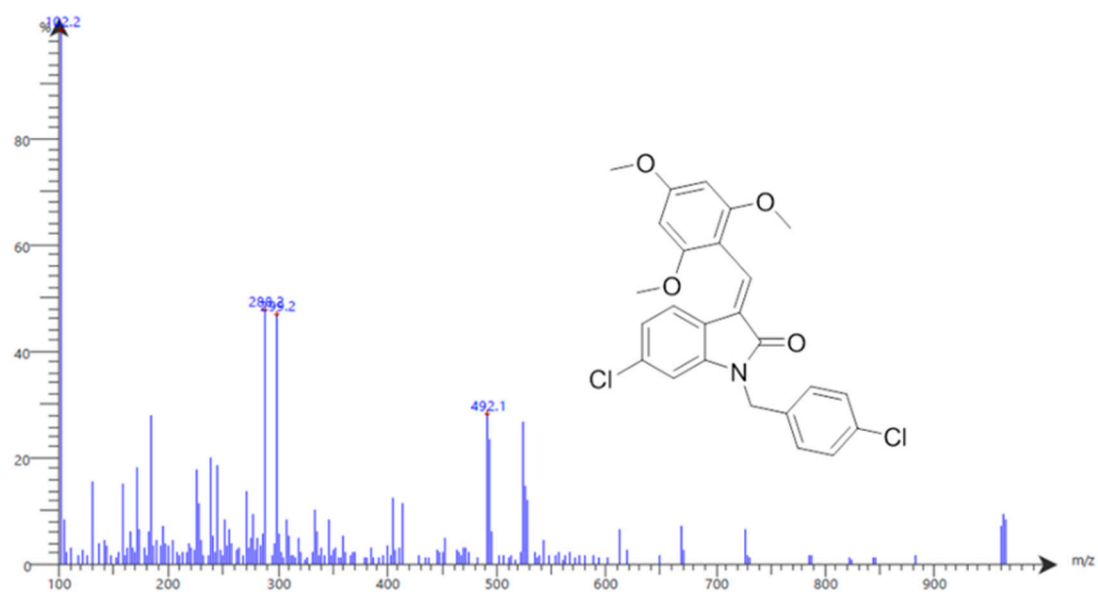


Figure S42. LC/MS spectrum of compound 7e.

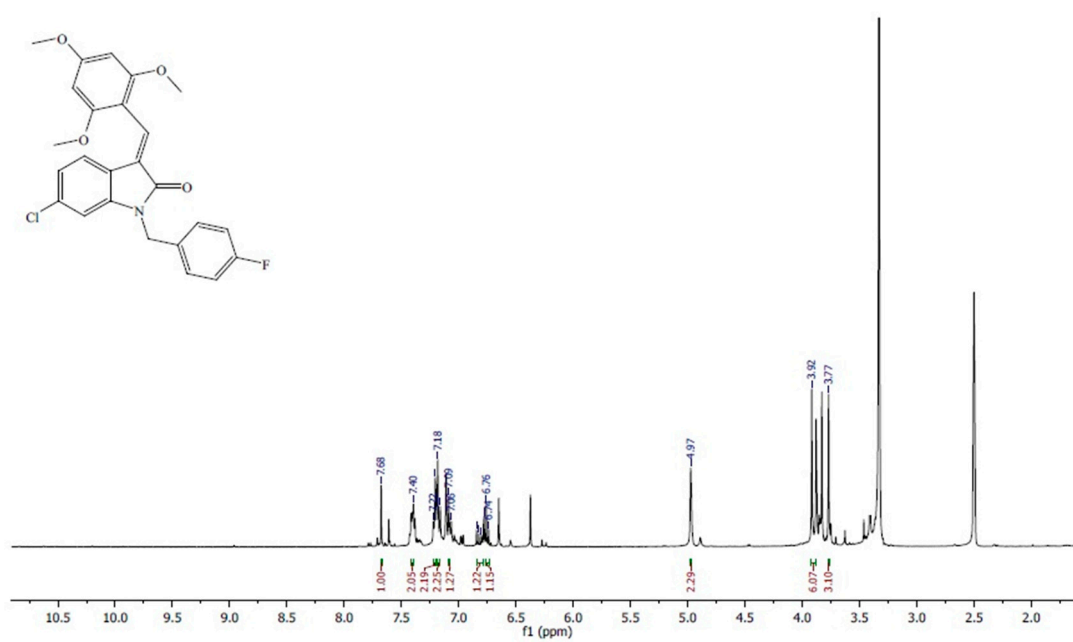


Figure S43. ¹H-NMR spectrum of compound 7f in DMSO-*d*₆ at 400MHz.

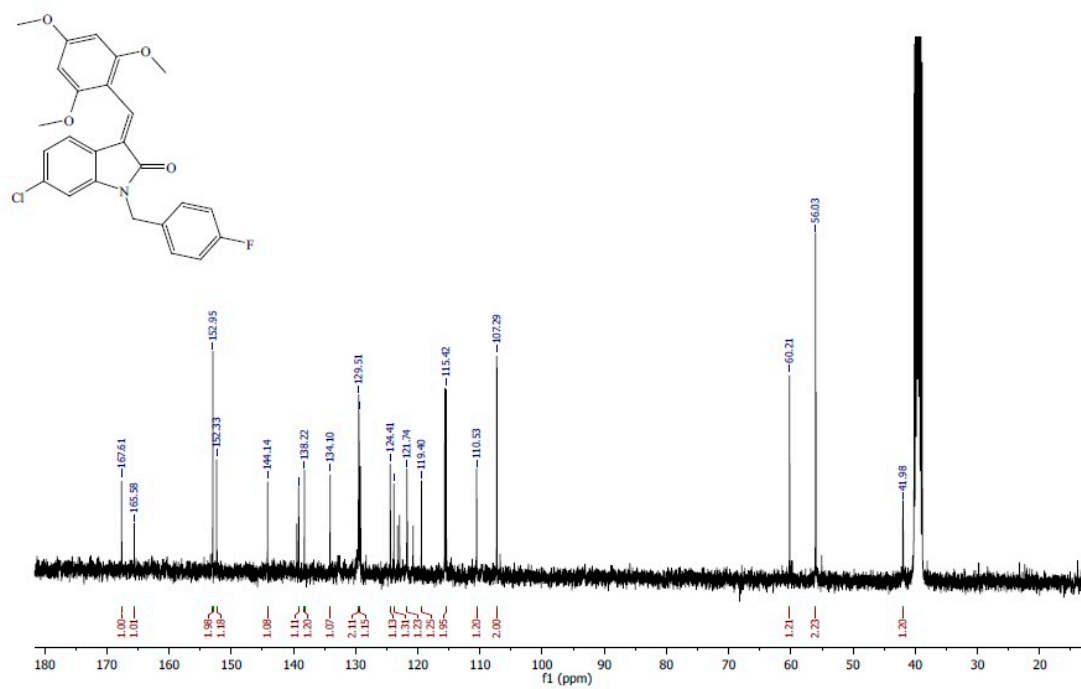


Figure S44. ¹³C-NMR spectrum of compound **7f** in DMSO-*d*₆ at 100MHz.

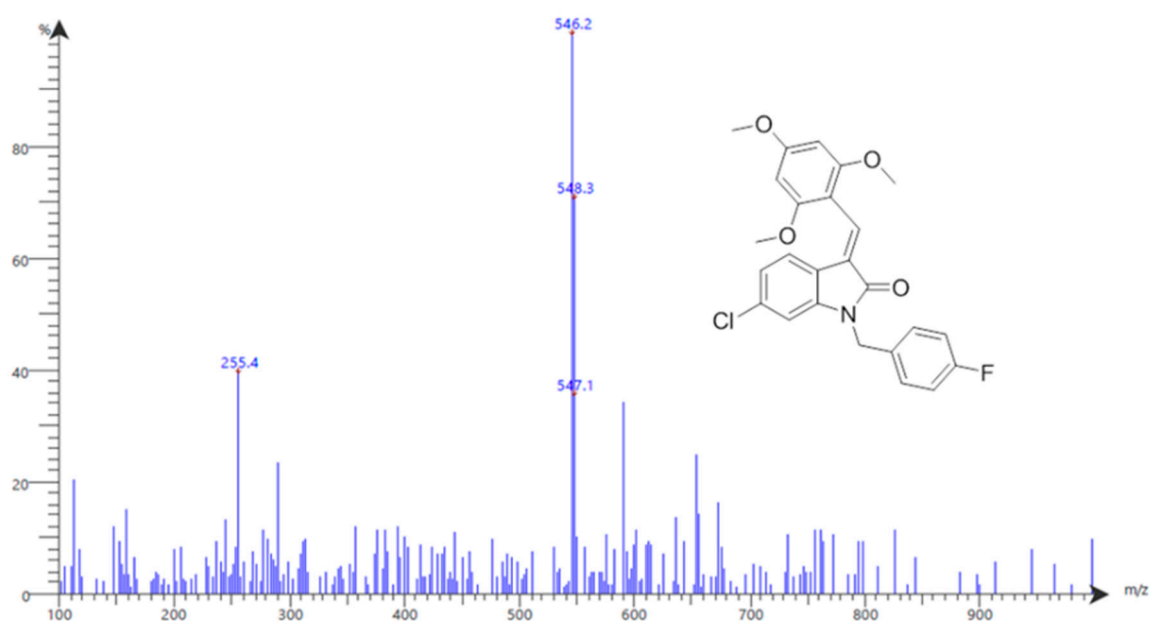


Figure S45. LC/MS spectrum of compound **7f**.

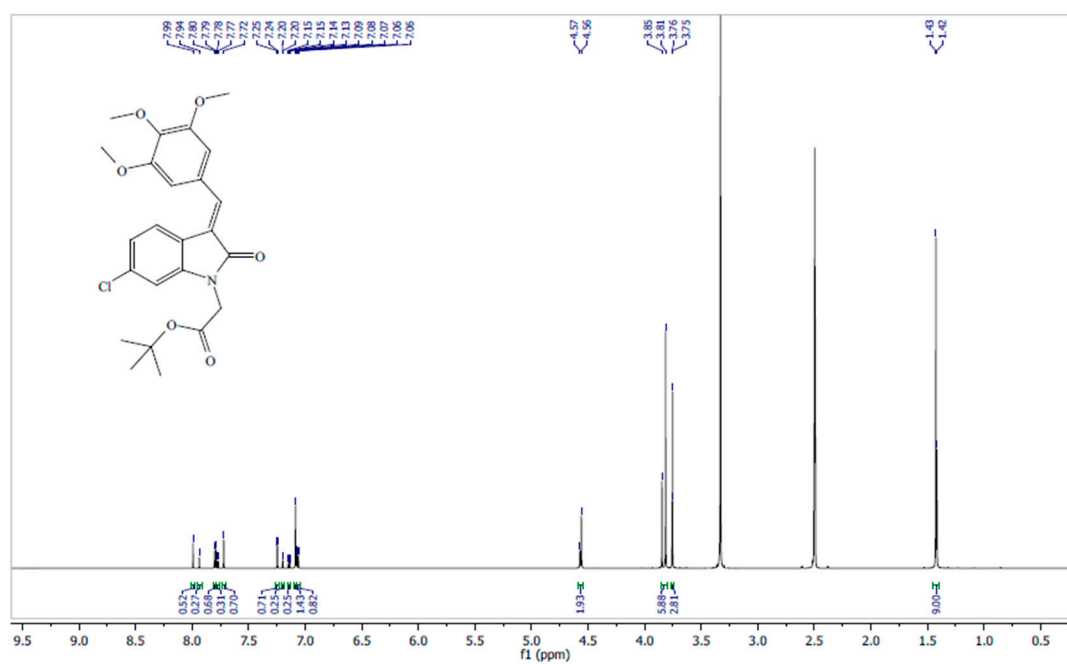


Figure S46. ¹H-NMR spectrum of compound **9a** in DMSO-*d*₆ at 400MHz.

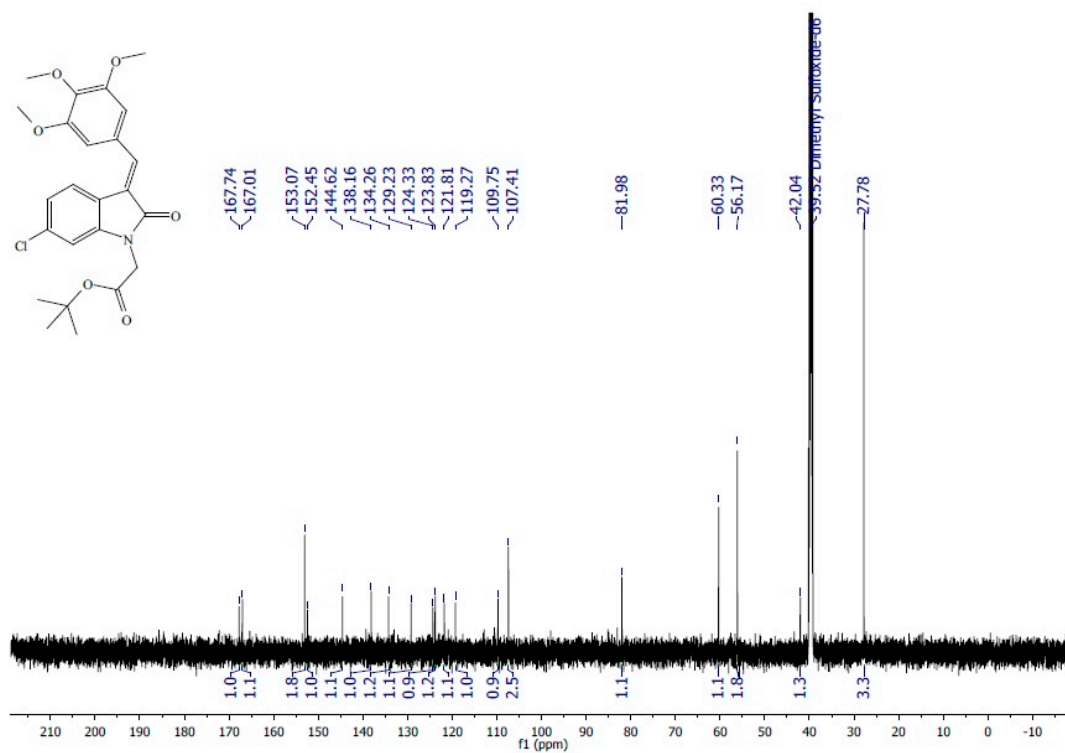


Figure S47. ¹³C-NMR spectrum of compound 9a in DMSO-*d*₆ at 100MHz.

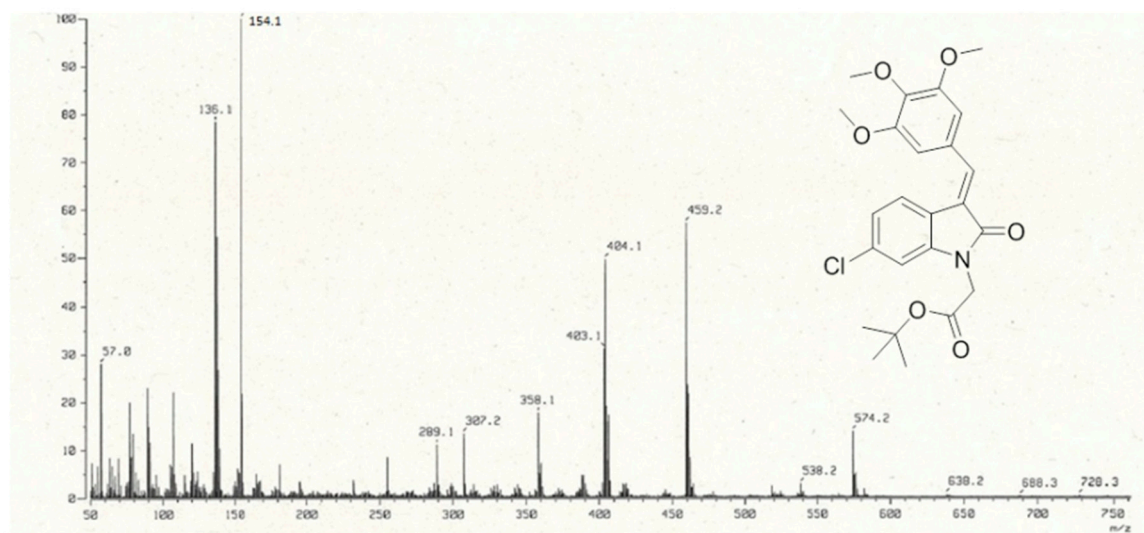


Figure S48. LC/MS spectrum of compound 9a.

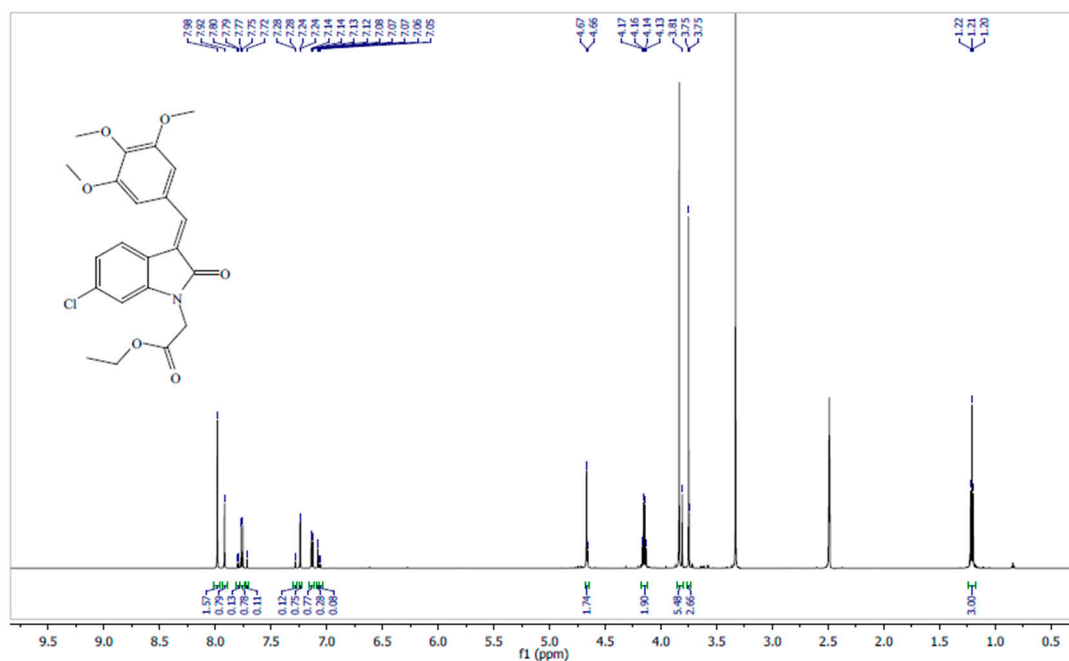


Figure S49. ¹H-NMR spectrum of compound **9b** in DMSO-*d*₆ at 400MHz.

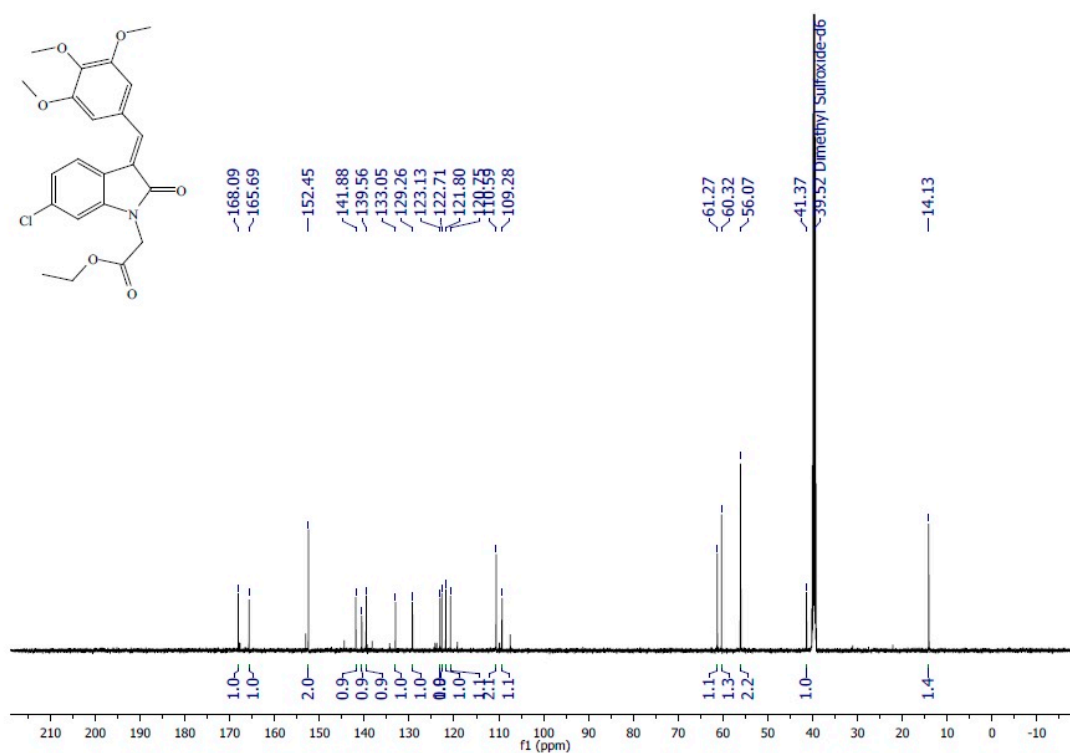


Figure S50. ¹³C-NMR spectrum of compound **9b** in DMSO-*d*₆ at 100MHz.

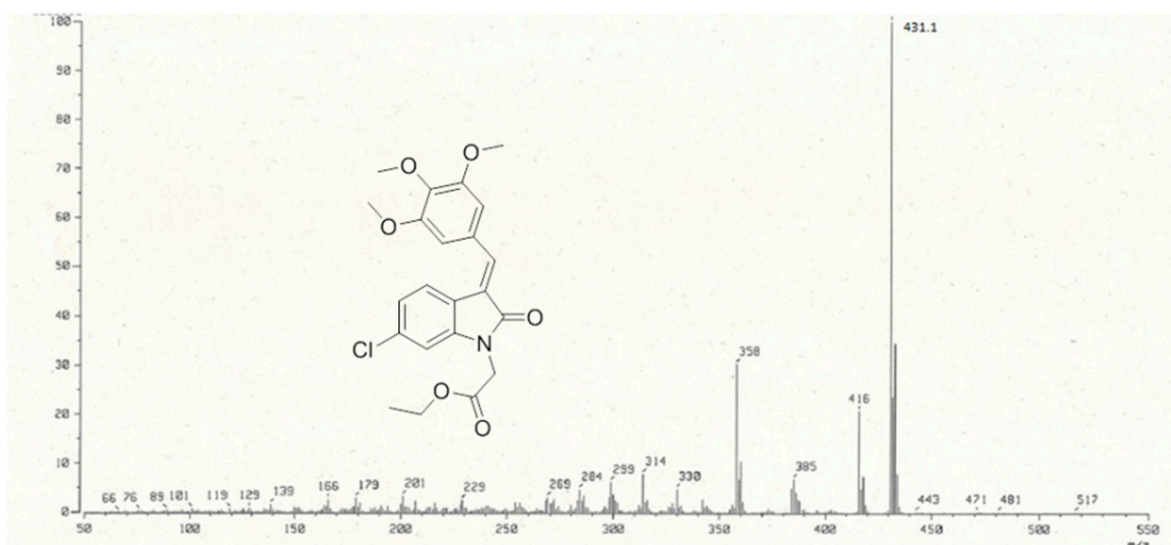


Figure S51. LC/MS spectrum of compound 9b.

Detailed Results

Tub

code	IC50	conc.	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
4b		100	2	91.7	30	0	30	1347	0	1347	539.47	9.98758	120
		10	1	69.4	30	0	30	4955	0	4955	539.47	36.73976	120
		1	0	42.5	30	0	30	9303	0	9303	539.47	68.97881	120
		0.1	-1	22.7	30	0	30	12517	0	12517	539.47	92.80961	120
	EC			0	30	0	30	16184	0	16184	539.47	120	120
code	IC50	conc.	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
CA4		100	2	92.3	30	0	30	1161	0	1161	501.27	9.264468	120
		10	1	75.8	30	0	30	3638	0	3638	501.27	29.03026	120
		1	0	54.5	30	0	30	6842	0	6842	501.27	54.59732	120
		0.1	-1	39.8	30	0	30	9057	0	9057	501.27	72.27243	120
	EC			0	30	0	30	15038	0	15038	501.27	120	120

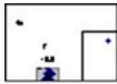
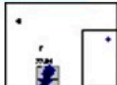
Researcher	: Dr. Mohamed badr	email : mohabadr180@gmail.com	mob. 01067157143
Assay	: Tubulin enzyme assay		
Samples	: 02 compounds		
Ref.	: ---		
Date	: 28-09-2021		
Reader	: Tecan-Spark reader		
Kit used	: Cloud-clone corp) SEB870Hu EIA Kit For Tubulin Beta (TUBb).		
Solvent	: DMSO		
Assay samples	: ---		


Lab Report

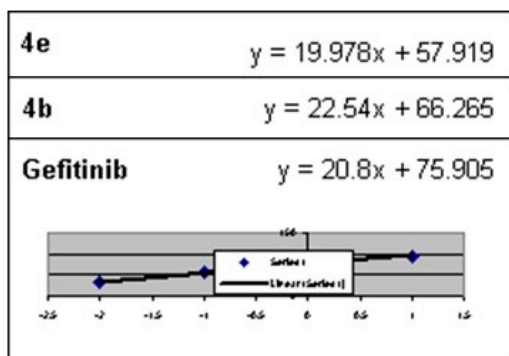
ser	Compound			Tubulin polymerization Inhibition	SD \pm
	ID	M.W g/mol	conc	IC50 uM	
1	4a	311.12		5.045	0.31
2	CA-4	316.34		1.384	0.09

Figure S53. Tubulin polymerization assay for compound 4a.

Detailed results

EGFR												
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
4e		10	1	78	30	0	30	22.06	0	22.06	3.33333	26.47203
		1	0	60	30	0	30	39.55	0	39.55	3.33333	47.46005
		0.1	-1	33	30	0	30	67.25	0	67.25	3.33333	80.70008
		0.01	-2	21	30	0	30	79.42	0	79.42	3.33333	95.3041
EC				0	30	0	30	100	0	100	3.33333	120
EGFR												
code	IC50	conc.ng/ml	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
4b		10	1	88	30	0	30	11.51	0	11.51	3.33333	13.81201
		1	0	69	30	0	30	31.42	0	31.42	3.33333	37.70404
		0.1	-1	40	30	0	30	59.96	0	59.96	3.33333	71.95207
		0.01	-2	23	30	0	30	77.13	0	77.13	3.33333	92.55609
EC				0	30	0	30	100	0	100	3.33333	120

code	IC50	conc.ng/ml	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Gefitinib		10	1	93	30	0	30	7.39	0	7.39	3.33333	8.868009
		1	0	82	30	0	30	18.34	0	18.34	3.33333	22.00802
		0.1	-1	56	30	0	30	44.12	0	44.12	3.33333	52.94405
		0.01	-2	32	30	0	30	68.13	0	68.13	3.33333	81.75608
	EC			0	30	0	30	100	0	100	3.33333	120



Researcher	: Dr.Mohamed Ibrahim	email: Mia06@fayoum.edu.eg	mob. 01025727434
Assay	: EGFR inh.assay		
Samples	: 02 compounds		
Cell lines	: ---		
Ref.	: ---		
Date	: 10-05-2021		
Reader	: ROBONIK P2000 ELISA READER	w/ 450 nm	
Kit used	: ---		
Solvent	: DMSO		

Lab Report

ser	Compound			EGFR	SD ±
	code	MW	conc. ug/ml	IC50 ug/ml	
1	4e			0.401	0.008
2	4b			0.19	0.004
3	Gefitinib			0.057	0.001

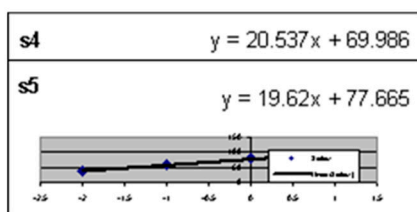
Figure S54. Epidermal growth factor receptor (EGFR) inhibition assay for compound 4b.

Detailed results

EGFR

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
4a		10	1	89	30	0	30	11.42	0	11.42	3.33333	13.70401	120
		1	0	72	30	0	30	27.55	0	27.55	3.33333	33.06003	120
		0.1	-1	50	30	0	30	49.65	0	49.65	3.33333	59.58006	120
		0.01	-2	27	30	0	30	72.51	0	72.51	3.33333	87.01209	120
EC				0	30	0	30	100	0	100	3.33333	120	120

code	IC50	conc.ng/ml	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
CMJ		10	1	93	30	0	30	6.56	0	6.56	3.33333	7.872008	120
		1	0	83	30	0	30	17.02	0	17.02	3.33333	20.42402	120
		0.1	-1	59	30	0	30	41.05	0	41.05	3.33333	49.26005	120
		0.01	-2	36	30	0	30	63.95	0	63.95	3.33333	76.74008	120
EC				0	30	0	30	100	0	100	3.33333	120	120



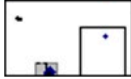

Researcher : Dr. Mohamed badr email : mohabadr180@gmail.com mob. 01067157143
 Assay : EGFR inh.assay
 Samples : 02 compounds
 Cell lines : ---
 Ref. : ---
 Date : 29-09-2021
 Reader : Tecan Spark Reader
 Kit used : ---
 Solvent : DMSO


Lab Report

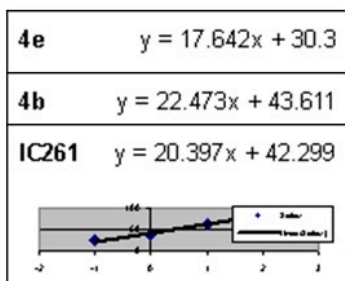
ser	Compound			EGFR	SD ±
	code	MW	conc. ug/ml	IC50 uM	
1	4a	311.12		0.106	0.0022
2	CJM 126	226.30		0.039	0.0008

Figure S55. Epidermal growth factor receptor (EGFR) inhibition assay for compound 4a.

Detailed results

CK1													
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
4e		100	2	65.47	30	0	30	0.634	0	0.634	0.0612	41.4379	120
		10	1	48.15	30	0	30	0.952	0	0.952	0.0612	62.2222	120
		1	0	30.23	30	0	30	1.281	0	1.281	0.0612	83.7255	120
		0.1	-1	12.64	30	0	30	1.604	0	1.604	0.0612	104.837	120
	EC			0	30	0	30	1.836	0	1.836	0.0612	120	120
													
code	IC50	conc.ng/ml	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
4b		100	2	88.62	30	0	30	0.209	0	0.209	0.0612	13.6601	120
		10	1	69.99	30	0	30	0.551	0	0.551	0.0612	36.0131	120
		1	0	35.62	30	0	30	1.182	0	1.182	0.0612	77.2549	120
		0.1	-1	25.16	30	0	30	1.374	0	1.374	0.0612	89.8039	120
	EC			0	30	0	30	1.836	0	1.836	0.0612	120	120
													

code	IC50	conc.ng/ml	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity	EC
IC261		100	2	85.59	30	0	30	0.287	0	0.287	0.0664	17.2892	120
		10	1	61.7	30	0	30	0.763	0	0.763	0.0664	45.9639	120
		1	0	36.8	30	0	30	1.259	0	1.259	0.0664	75.8434	120
		0.1	-1	25.9	30	0	30	1.476	0	1.476	0.0664	88.9157	120
	EC			0	30	0	30	1.993	0	1.993	0.0664	120	120
													



Researcher	: <u>Dr. Mohamed Ibrahim</u> email: Mia06@fayoum.edu.egmob.01025727434
Assay	: CK1 assay
Samples	: 02 compounds
Cell lines	: ---
Ref.	: ---
Date	: 20-05-2021
Reader	: ROBONIK P2000 ELISA READER <u>wl</u> 450 nm
Kit used	: ---
Solvent	: DMSO

Lab Report

<u>ser</u>	Compound			Casein kinase I CK1	SD ±
	code	MW	conc. <u>ug/ml</u>	IC50 <u>ug/ml</u>	
1	4e			13.08	0.66
2	4b			1.92	0.09
3	IC261			2.385	0.12

Figure S56. Casein kinase I (CK1) inhibition assay

detailed
results

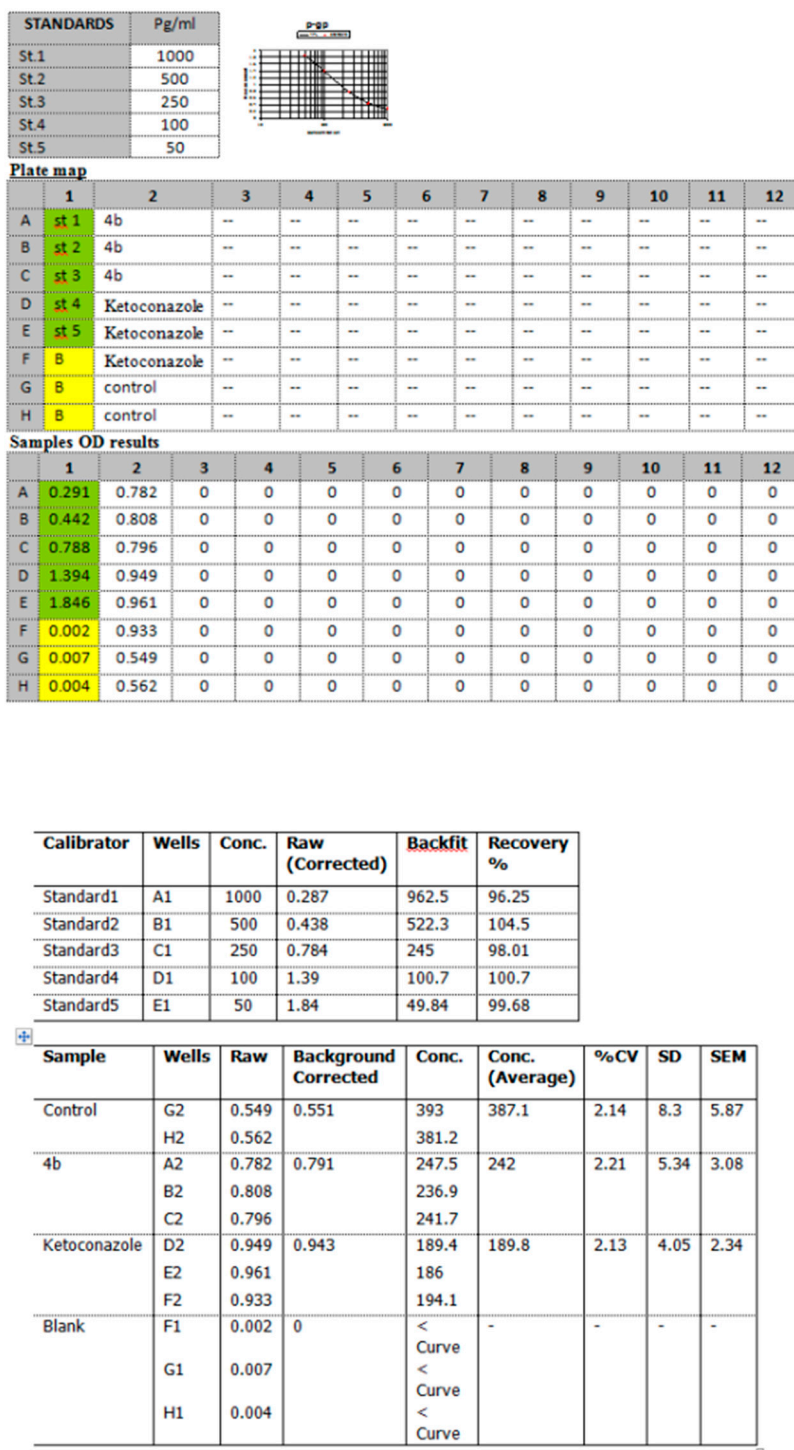


Figure S57. P-gp substrate assay.

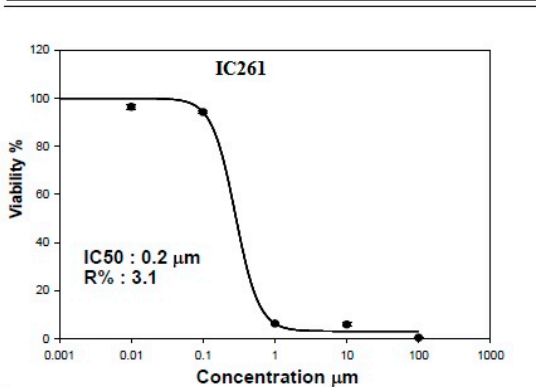
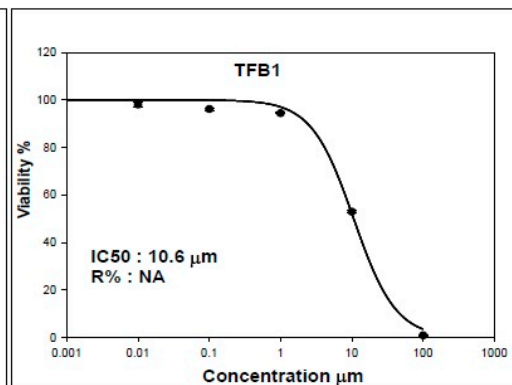
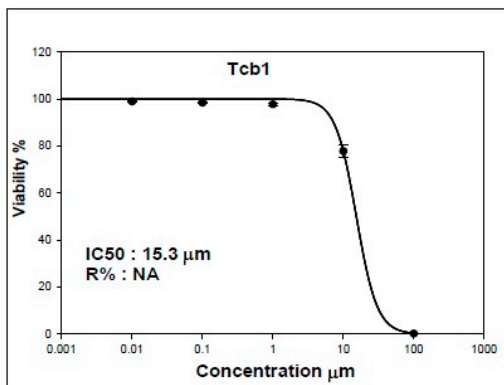
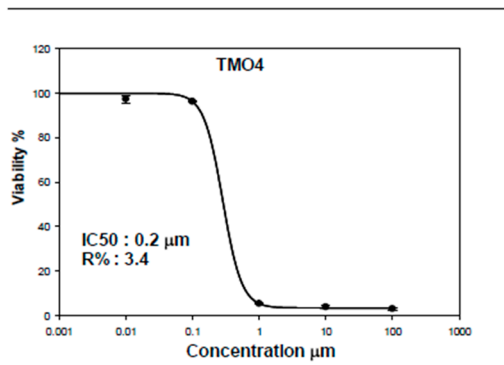
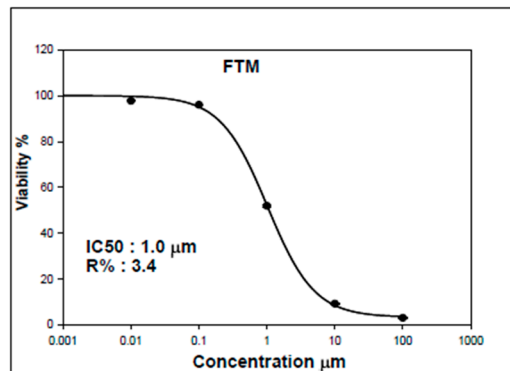
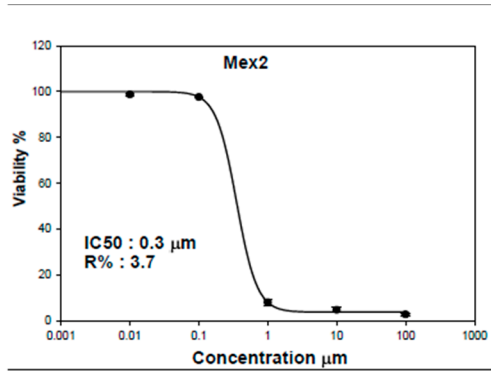


Figure S58. IC₅₀ of compounds **4a** (IC₂₆₁), **4b**, **4d**, **4e**, **6e** and **6f** against COLO-205 using SRB assay.

Researcher	: Dr. Mohamed badr	email: mohabadr180@gmail.com	mob. 01067157143
Assay	: MTT cytotoxicity assay		
Samples	: 01 compound.		
Cell lines	: ----		
Ref.	: ----		
Date	: 17-10-2021		
Reader	: BIOLINE ELIZA READER	wl 450 nm	
Kit used	: SIGMA chemicals		
Solvent	: DMSO		

Lab Report

*Cytotoxicity results

Ser	Sample		cytotoxicity IC ₅₀ uM	SD ±
	code	M.W g/mol	A549	
1	4a	311.12	28.4	1.52
2	4b	345.08	9.5	0.51
***	5-FU	130.078	15.6	0.83

Figure S59. Cytotoxicity assay and IC₅₀ of compounds **4a**, **4b** and **5-FU** against A549 cell line.

Cell culture Protocol

Cell Line cells were obtained from American Type Culture Collection , cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were from Sigma, or Invitrogen.

Plate cells (cells density $1.2 - 1.8 \times 10,000$ cells/well) in a volume of 100µl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before the MTT assay .

Cell culture protocol

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin 0.53 mM EDTA solution to remove all traces of serum which contains Trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C for 24 hrs.
- 8-After treatment of cells with the serial concentrations of the compound to be tested incubation is carried out for 48 h at 37°C ,then the plates are to be examined under the inverted microscope and proceed for the MTT assay

MTT – Cytotoxicity assay protocol

The MTT method of monitoring in vitro cytotoxicity is

well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 106 cells/cm². Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Reconstitute each vial of MTT [M-5655] to be used with 3 ml of medium or balanced salt solution without phenol red and serum. Add reconstituted MTT in an amount equal to 10% of the culture medium volume.
3. Return cultures to incubator for 2-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should be consistent when making comparisons.
4. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of MTT Solubilization Solution [M-8910] equal to the original culture medium volume.
5. Gentle mixing in a gyratory shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely dissolve the MTT formazan crystals.
6. Spectrophotometrically measure absorbance at a wavelength of 570 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 450 nm measurement. Tests performed in multiwell plates can be read using the appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvetts for spectrophotometric measurement.

researcher

assay

Date

cells

Dr. Moh. Badr

MTT

17-Oct

A549

	Blank	CC	Sample No. 4a/A549					Sample No. 4b/A549				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 Eia reader

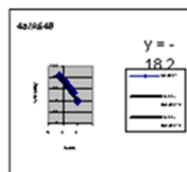
Wave length: 450 nm

Reference: 630nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.549	0.215	0.288	0.339	0.402	0.454	0.167	0.245	0.298	0.343	0.379
B	0	0.533	0.203	0.283	0.347	0.392	0.462	0.172	0.237	0.303	0.346	0.388
C	0	0.561	0.217	0.292	0.345	0.398	0.457	0.178	0.242	0.306	0.352	0.394
mean	0	0.548	0.2117	0.2877	0.3437	0.3973	0.4577	0.1723	0.241	0.3023	0.347	0.387
%			38.649	52.526	62.751	72.55	83.567	31.467	44.07	55.204	63.36	70.663

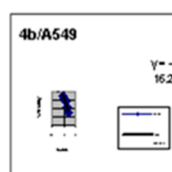
4a/A549

log conc.	% viability
2	38.6
1.398	52.5
0.796	62.8
0.193	72.6
-0.41	83.6



4b/A549

log conc.	% viability
2	31.47
1.3979	44.07
0.7959	55.2
0.1931	63.36
-0.409	70.66



IC50=

IC50=

	Blank	CC	Sample No. 5-FU/A549					Sample No.				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM					

ROBONIK P2000 Eia reader

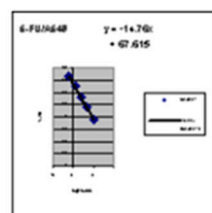
Wave length: 450 nm

Reference: 630nm

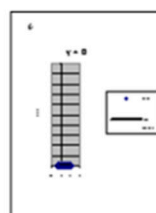
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.488	0.178	0.228	0.272	0.324	0.367					
B	0	0.475	0.193	0.236	0.279	0.308	0.356					
C	0	0.505	0.184	0.234	0.265	0.319	0.358					
mean	0	0.489	0.185	0.2327	0.272	0.317	0.3603	0	0	0	0	0
% viability			37.807	47.548	55.586	64.782	73.638	0	0	0	0	0

5-FU/A549

log conc.	% viability
2	37.8
1.398	47.5
0.796	55.6
0.193	64.8
-0.41	73.6



log conc.	% viability
2	0
1.3979	0
0.7959	0
0.1931	0
-0.409	0



IC50=

IC50=

Figure S60. Raw data of cytotoxicity assay and IC₅₀ of compounds **4a**, **4b** and **5-FU** against A549 cell line.

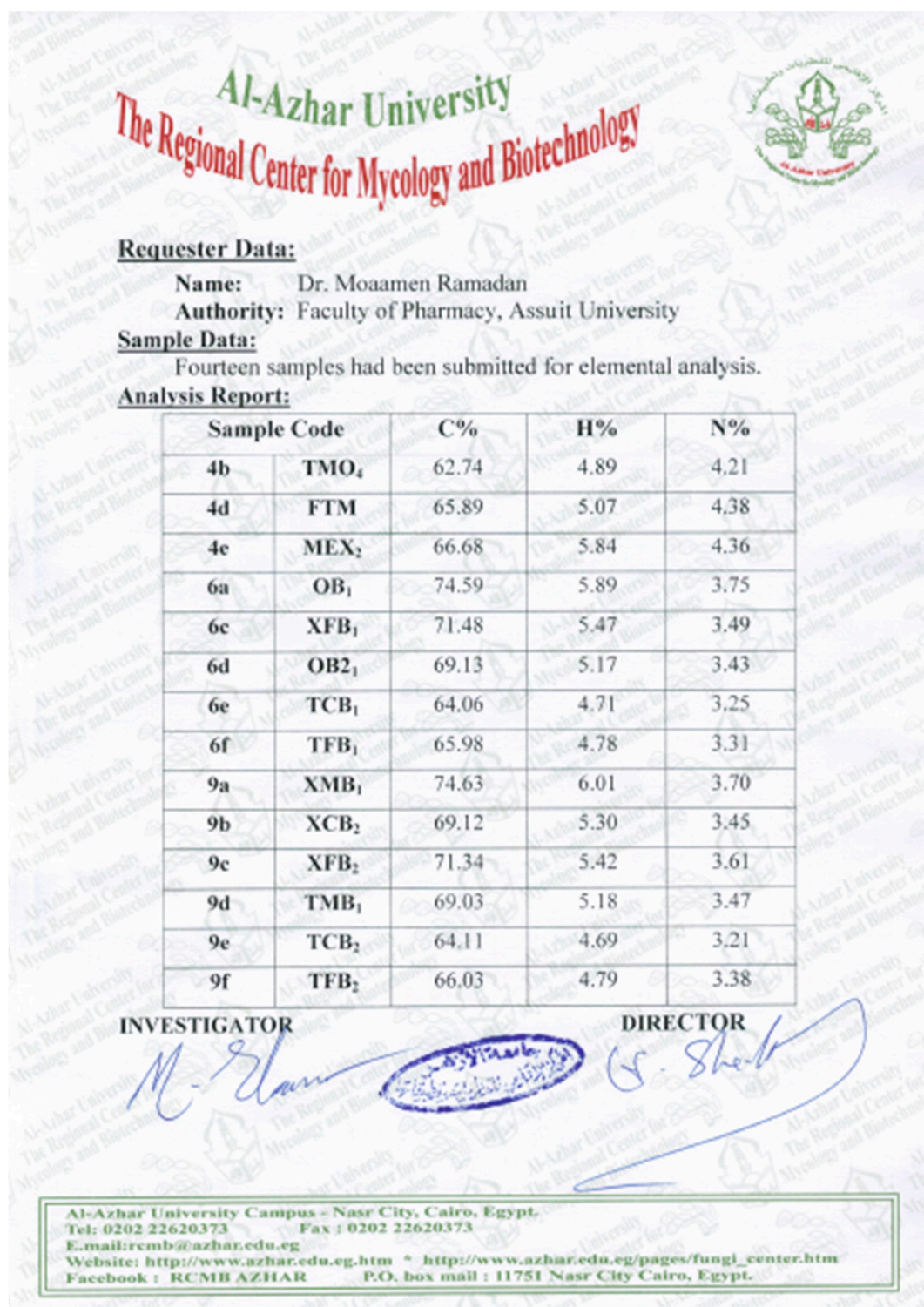


Figure S61. elemental analysis data.